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ERZSÉBET KÖVES, L. SZALAY, F. ZSOLDOS

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THE CHARACTERISTICS OF ASPARTATE TRANSAMINASE ENZYME IN TOBACCO TISSUE CULTURES

I. GAÁL and ERZSÉBET KÖVES

Department of Plant Physiology, Attila József University, Szeged
(Received September 10, 1982)

Abstract

The optimal temperature of the activity of the aspartate transaminase enzyme obtained from tobacco tissue cultures is 53°C at 8.5 pH. The activation entalpy of the reaction is 33.5 KJ, Km = $1.15.10^{-5}$M on aspartate. The reaction does not require pyridoxal phosphate. Ca^{2+}, Sr^{2+}, Ba^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Pb^{2+}, Mn^{2+} do not influence the enzyme activity.

Cd^{2+}, Hg^{2+}, Zn^{2+}, Cu^{2+}, Ag^{+}, semicarbazide hydroxylamine, parachlor-mercurybenzoate inhibit the reaction. There is correlation between the inhibitory effect and the electron structure of the metal ions. Aspartate transcminase activity is measurable in every culture, while in the case TRP substrate transamination is only detectable in the so-called habituated cultures.

Key words: Aspartate transaminase, tryptophan-transaminase, tobacco callus, ion effect, enzyme inhibition, *Nicotiana tabacum* cv. Xanthi.

Abbreviations: ASP = aspartate, DNFH = dinitrophenylhydrazine, GLU = glutamic acid, GOT = glutamic acid-oxaloacetic acid-transaminase, IAA = -indole-3-acetic acid, PCMB = parachlor-mercurybenzoate, TRP=tryptophan.

Introduction

The aspartate transaminase (SMITH and WILLIAM, 1951) is one of the mostly studied enzymes among the plant-transaminases. It participates in the metabolism of amino acids (FOWDEN, 1967), takes part in the pathway of the glyoxylic acid (TOLLBERT and YAMAZAKI, 1969), the C_4-reaction pathway, and the intercellular transport of the metabolites (HATCH, 1971), It catalyzes the transformation of tryptophan in to indole-3-pyruvic acid from which indole-3-acetic acid is formed (GORDON, 1961; LARSEN, 1967; SCHNEIDER et al., 1972; HERKLOSS and LIBBERT, 1976). Certain metal ions influence the activity of the enzyme (HAPPOLD and TURNER, 1957; NADKARNI and KAMALA, 1962; PATWARDHAN, 1960; VERJEE and EVERED, 1969). Latter authors studied the influence of metal ions on the enzyme in different plants. The obtained results are contradictory in many cases. In our opinion the contradictions cannot be fully explained by the variations in species. Examination of certain substances and physiological processes in plant segments is very complicated due to the differentiation of the cells, since the tissues of various structures may react differently from the meristematic cells. Morphologically and physiologically the cell- and tissue cultures are homogeneous systems. Their cells are dedifferentiated. The keeping under sterile conditions of the tissue cultures is also easier and more controllable, therefore, it seemed reasonable to study a few basic characteristics of this important enzyme in tobacco callus culture. Since the role of the enzyme in the auxin synthesis also belonged to our range of interest, in one series habituated callus cultures capable of auxin synthesis were used, and normal callus cultures not growing without auxin were used in the other experiments. As the enzyme transforms aspartate with a rate of nearly two ordes

higher than it does in the case of tryptophan (FOREST and WIGHTMAN, 1972) it seemed reasonable to study certain features of the aspartate transaminase, enzyme.

Materials and methods

Cultivation of tobacco callus

Nicotiana tabacum cv. Xanthi pith parenchyma callus cultures were used in our experiments, grown on Murashige-Skoog media (MURASHIGE and SKOOG, 1962, SKOOG and LINSMAIER, 1965). The habituated cultures were grown auxin-free media, while the heterotrophic calluses were cultivated on media containing 3 mg/l IAA and 0.1 mg/l 2.4-D. The media contained 0.04 mg/l kinetin in every case. The cultures were grown in Conviron growth chambers at 25°C under light of 1.23 mWatt/cm².

Extraction of transaminase enzyme

The 3 weeks old tobacco calluses were homogenized in mortar at 0—5 °C in 0.05 M Tris-HCl buffer (MATHERON and MOORE, 1973). The EDTA concentration of the buffer was 0.01 M, that of mercaptoethanol was 0.001 M. The fresh weight and buffer ratio of the callus was 1:3. The homogenate was centrifuged in a cooling centrifuge for 30 min. at 1000 g and 5 °C. The supernatant was saturated with ammonium sulfate up to 35%. After standing overnight centrifugation according to the above described procedure was carried out once more. This time the supernatant was saturated with ammonium sulfate up to 80%. After standing overnight and a repeated centrifugation the precipitate was resolved in 0.05 M Tris-HCl buffer (pH8.5) it was dialysed against 0.05 M Tris-HCl buffer in a refrigerator (5 °C) for one day. The protein extract was centrifuged as described above; concentrated with Aquacide II, centrifuged again and the supernatant was used for our studies.

Measuring of aspartate transaminase enzyme activity

The determination of enzyme activity were carried out with the method of REITMAN and FRANKEL cf. Sós (1974). The composition of GOT substrate was 1 mM α-ketoglutaric acid + 0.2 M L-aspartic acid in 0.05 M Tris-HCl buffer (pH 8.5). One ml of GOT substrate was preincubated at 37 °C for 5 min, then mixed with 200 µl protein extract (1.7 mg/ml protein). After 20 min. 1 ml of dinitrophenylhydrazine after further 20 min. 10 ml of NaOH was added. The absorption of the solution was measured with spectrophotometer at 500 nm after 5 min. Instead of the enzyme, a mixture containing 0.2 ml H_2O was used as comparative solution.

Measuring of tryptophan transaminase enzyme activity

Applying the method of LIU (1978) several disturbing circumstances occurred (GAÁL and KÖVES, 1981) during the course of the activity measurements by spectrophotometry, therefore the TRP transaminase activity was followed by the quantitative measuring of GLU end-product. The incubation mixture was composed of 0.01 M EDTA, 0.03 M TRP, 0.0002 M pyridoxal phosphate, 0.855 g enzyme protein in 10 ml borate buffer (pH 8.5). The mixture was taken into two halves and icubated at 45 °C for 15 min. Then 0.5 ml buffer was added to one of the mixtures, and 0.5 ml 0.06 Mμ-ketoglutaric acid-containing buffer to other. After 1 h the reaction was stopped with 3 drops of cc. HCl solved in 2 ml of ethanol. The solution was saturated at low temperature, centrifuged and the supernatant was evaporated in a vacuum, then dissolved in 1 ml buffer. GLU was determinded with the help of an AAA 881 Mikrotechna (product of Czehoslovakia) automatic amino acid analyser. Na-citrate (pH 3.25; 4.25; 5.28) was used as buffer. The samples were evaporated to dryness and dissolved in 1 ml buffer.

Determination of protein

Protein was determined by the method of LOWRY et al. (1951).

Results

pH-DEPENDENCY OF THE ENZYME ACTIVITY

The activity of the enzyme was measured between pH 6—11. The changes in enzyme acitivity in the function of pH are described by a maximum curve. The point of the maximum is at pH 8.5 (Fig. 3).

DEPENDENCE OF ENZYME ACITIVITY ON THE TEMPERATURE

The temperature-dependency of the aspartate transaminase enzyme acitivity was studied between 19—62 °C. The activity-maximum was at 53 °C (Fig. 1). Though activity was highest at this temperature, the rate of the reaction was only constant for 30 min. Therefore, the measuring were made at 37 °C, where the rate of substrate-transformation was lower, however, it showed no changes for several hours. The activation entalpy of the reaction was 35.5 KJ, obtained by the graphic presentation of the linear form of the Arrhenius equation (Fig.2).

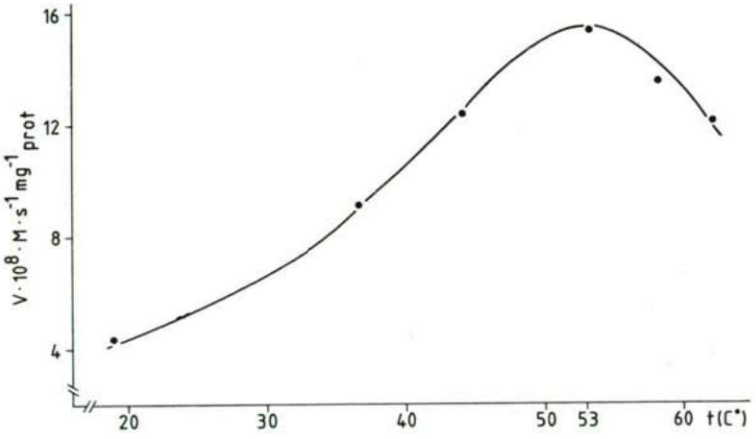

Fig. 1. Temperature-dependency of the transamination reaction. Ordinate: enzyme activity. Abscissa: temperature. Composition of the reaction mixture is described in „Materials and Methods"

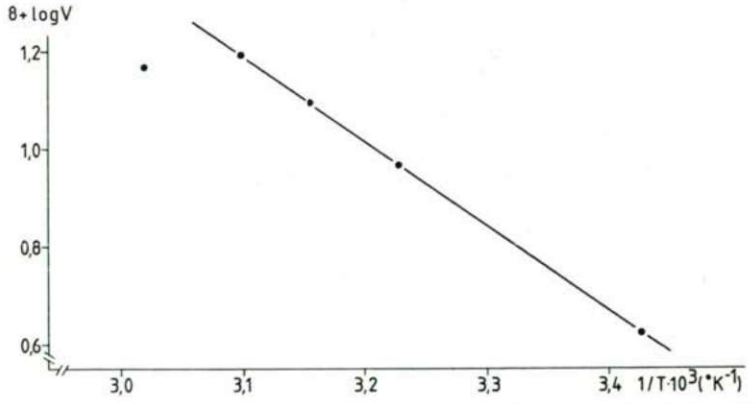

Fig. 2. Determination of the activation entalpy from the linear form of the Arrhenius equation. The ordinate shows the acitivity, which is: M $s^{-1}mg^{-1}$ protein.

ASP AND TRP TRANSAMINASE ENZYME ACITIVITIES

The acitivity of aspartate transaminase at 37 °C and pH 8.5 was $9.10^{-8}M.s^{-1}$ mg^{-1} protein, while that of TRP transaminase enzyme was $0.58.10^{-8}M.s^{-1}.mg^{-1}$ protein. According to our measurings the activity of the enzyme obtained from the tobacco callus was not stimulated by pyridoxal phosphate (Table 3).

Table 1. Changes in the specific acitivity of the aspartate transaminase enzyme and the rate of inhibition in presence of various metal ions.

	Initial concentration of ion (μM)	Concentration of the formed pyruvate 10^{-4}M	Inhibition %
control	0·00	3.83	0.00
Hg^{2+}	1.66	3.83	0.00
	12.50	3.22	26.00
	50.00	1.98	49.00
	100.00	1.66	57.00
	200.00	0.78	80.00
Cd^{2+}	1.66	3.83	0.00
	12.50	2.88	25.00
	50.00	1.74	55.00
	100.00	1.05	73.00
	200.00	0.52	87.00
Zn^{2+}	1.66	3.57	7.00
	12.50	3.57	7.00
	50.00	2.61	32.00
	100.00	2.18	44.00
	200.00	1.39	64.00
Cu^{2+}	1.66	3.66	5.00
	12.50	3.66	5.00
	50.00	3.50	7.00
	100.00	2.87	26.00
	200.00	2.26	31.00
	400.00	0.87	88.00
control	0.00	4.35	0.00
Ag^{+}	1.66	4.01	6.00
	3.13	3.92	11.00
	6.25	3.66	17.00
	12.50	2.45	44.00
	25.00	2.17	51.00

The composition of the reaction mixture is described in "Materials and Methods".

KINETICS OF THE TRANSAMINASE REACTION

The linear form of the Lineveawer-Burk function was used for the determination of the kinetic parameters (Fig. 4). In the case of aspartic acid substrate a value of $K_m = 1.11.10^{-5}$M; in the case of α-ketoglutaric acid $K_m = 5.8.10^{-5}$M value was received.

INFLUENCE OF CATIONS ON THE ASPARTATE TRANSAMINASE ENZYME ACTIVITY

For studies on the ion effect the 1 ml GOT substrate was preincubated with 0.5 ml solution containing various ions, and then 0.2 ml enzyme solution was added. The

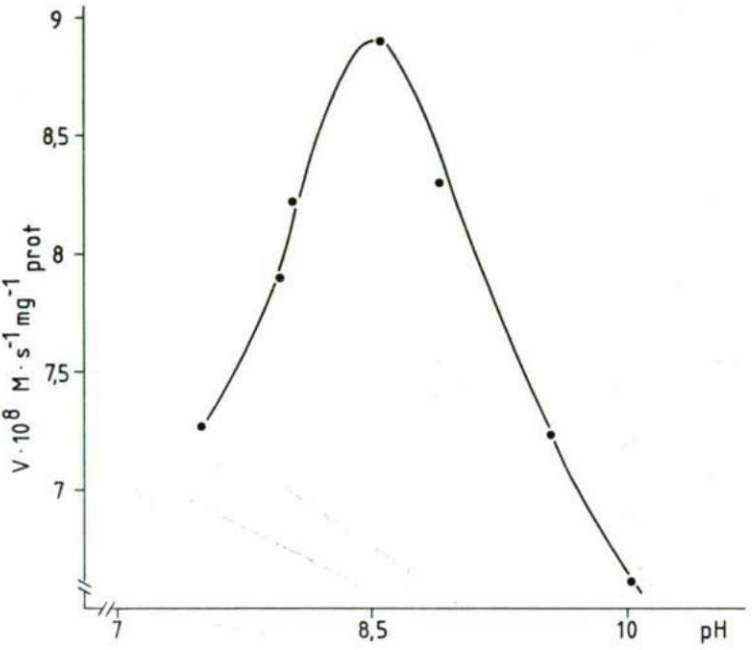

Fig. 3. pH-decencency of the transamination reaction.

Table 2. Influence of PCMB, hydroxylamine and semicarbazide on the aspartate transaminase enzyme activity.

PCMB μM	Spec. act. 10^{-1}M s^{-1} mg^{-1} protein	Hydroxylamine and semicarbazide μM	Spec. activity	
			hydroxylamine	semicarbazide
			μM $s^{-1}mg^{-1}$ protein	
0.0	8.6	0.0	8.2	8.2
2.0	8.2	100.0	6.4	5.1
4.0	7.8	200.0	5.7	3.6
6.0	2.7	300.0	5.3	2.3
8.0	1.6	400.0	5.0	1.3

The composition of the reaction mixture is described in „Materials and Methods"

reaction was followed for 30 min. The effect of 13 ions was studied in the experiments: Ca^{2+}, Sr^{2+}, Ba^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Ba^{2+}, Pb^{2+}, Cd^{2+}, Hg^{2+}, Zn^{2+}, Cu^{2+}, Ag^{+} (Fig. 5).

From the listed ions the last five decreased the rate of aspartic acid transamination. Table 1 shows the initial concentrations of the ions, with the inhibition pertaining to them.

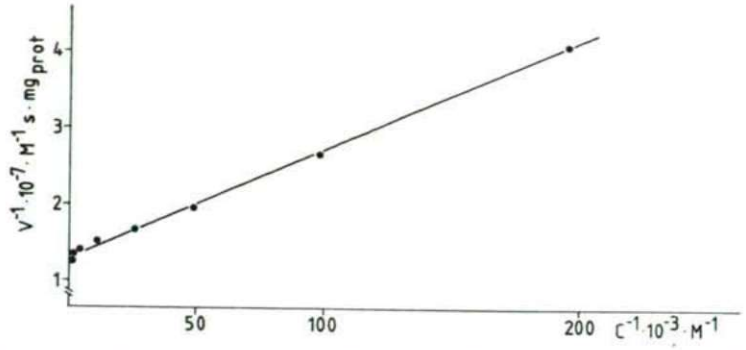

Fig. 4. Changes in activity according to Lineveawer-Burk. The reciprocity of the activity is plotted against the reciprocity of the aspartic acid initial concentrations.

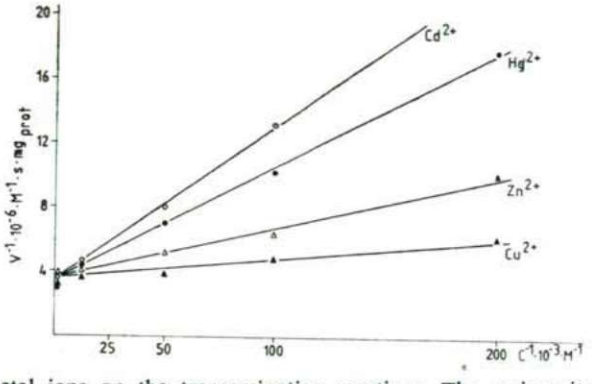

Fig. 5. Effects of metal ions on the transamination reactions. The reciprocity of the activity is plotted against the initial concentrations of metal ions.

Table 3. The influence of pyridoxal phosphate with different initial concentrations on aspartate transaminase acitivity.

Pyridoxal phosphate μM	Absorption
0.00	0.161
4.00	0.140
6.25	0.150
12.50	1.160

Discussion

It can be seen from the results that the rate of TRP transamination is lower by two orders than that of aspartate transformation. This is in accordance with the expectations since through the transamination of TRP IAA develops with low intensity (SCHNEIDER et al., 1972) both in the tissues and in vitro. The TRP transaminase enzyme activity could not be measured in the auxin heterotrophic culture, which is in coordination with the rather low IAA-level if this culture, also known by us (KÖVES et al., 1981). On the contrary, the aspartate transaminase enzyme was observed to be equally active in both cultures.

Our earlier gas-chromatographic determinations indicate that the auxin heterotrophic callus also contains IAA — although in lower amount — as does the autotrophic (habituated) culture (KÖVES et al., 1981). Therefore, it is assumed that in the heterotrophic culture IAA is not formed by indolepyruvic acid pathway. The possible alternatives are: the synthesis starting with TRP-decarboxylase or the forming of IAA in a non-enzymatic way. LIU et al. (1978), however, did not find TRP decarboxylase activity in Nicotiana calluses, and could not demonstrate tryptamine as an intermedier. Others, e. g. PHELPS and SEQUERIRA (1968) demonstrated IAA-synthesis throug h tryptamine in the cell-free extract of tobacco terminal buds; and SIMTH (1977) found tryptamine in Nicotiana leaves. According to SATOH and ESHASHI (1982) TRP does not only transform into IAA on the effect of a decarboxylase in cocklebour seeds, but doing so it also increases the production of ethylene.

LIU et al. (1978) measured the TRP transaminase activity in tumorous and nontumorous calluses, and experienced that in the tumorous tissue culture pyridoxal phosphate did not influence the enzyme activity, while the enzyme from the mutants showed pyridoxal phosphate-dependency. The enzyme activity of the nontumorous tobacco calluses studied by us did not increase in the presence of pyridoxal phosphate even when the enzyme extract was previously filtered on Sephadex G-25.

According to FOWDEN (1965), as well as MATHERON and MOORE (1973) semicarbazide and hydroxylamine react with the aldehyde groups, and inhibit the pyridoxal phosphate-dependent reactions, while the PCMB is a sulfhydryl reagent. According to our measurings these compounds also inhibit enzyme activity in the presence of pyridoxal phosphate (Table 2). Since there is also inhibition despite the co-enzyme bound to the apo-enzyme, it is our assumption that the compounds in question effect another active centre of the enzyme.

From the 13 ions studied by us only the Cd^{2+}, Hg^{2+}, Zn^{2+}, Cu^{2+}, and Ag^{+} influenced the enzyme activity and acted as inhibitors. These ions are sulfhydryl reagents, therefore it is probable that they exert their inhibitory effect by blocking the SH-group of the enzyme. Their common characteristic is that on their external electron shell the electron arrangement is $s^2p^6d^{10}$, form which the Cu^{2+} froms and exception, nevertheless, it also fulfils this condition in a reduced state.

The electron arrangement on the external shell of the ions, the diameters of ions of sheath of solvents the compactness of the ions largely influence the interrelationship with the active centers of the enzyme. The complete or partial conformity of these features may produce similar effect on the enzyme activity. Taking them into consideration, the explanation of the contradictions found in the literature, as well as the proposal of experiments may both become easier.

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Address of the authors: DR. IMRE GAÁL and DR. ERZSÉBET KÖVES Attila József University Department of Plant Physiology Szeged, P.O. Box 654. Hungary

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ENHANCEMENT OF THE DEGREE OF DROUGHT-RESISTANCE IN VARIETIES BY CROSSING AND SELECTION ON THE BASIS OF PROLINE-CONTENT

G. PÁLFI, ZSÓFIA PÁLFI and L. PINTÉR
Department of Plant Physiology, Attila József University, Szeged;
Institute of Plant Physiology, Biological Research Center, Hungarian Academy of Sciences, Szeged;
Cereal Research Institute, Szeged
(Received July 15, 1982)

Abstract

It has been determined that from the cultivated varieties (inbred lines and hybrids) belonging to one species, that has the highest level of drought-resistance which accumulates the largest amount of free proline in the isolated leaves, as the consequence of the lethal water deficiency developing gradually within three days. From 36 types of inbred corn lines the isolated leaves of the corn line "Le 60" showed the highest proline concentration developing on the effect of lethal — thus the same internal — water deficiency, provoked by live-wilting. An artificial population was established by the three way crossing (TC) of this line and two related ones, and for a period of two years proline selection was carried out individually on the offspring plants. Studies on the 64 individuals of the „S_o" and „S_1" generations demonstrated that the drought-resistance of the inbred lines can be increased significantly by crossing and individual selection, on the basis of the degree of proline accumulation.
Key words: *Zea mays*, drought-resistance, proline-content

Introduction

Several researchers have found that on the effect of strong water deficiency the degree of drought-resistance of certain varieties of soft-stalked plant species is directly proportional to the proline concentration synthetized and accumulated in the leaves, if the same level of „internal water deficiency" is produced in the plants (BLUM and EBERCON, 1976; BRITIKOV, 1975; GOAS, 1966; HUBAC and GUERRIER, 1972; LEWITT, 1972; MALI and MEHTA, 1977; SASHIDHAR et al. 1977; SINGH et al. 1972; SRINIVASA, 1977; VAN DE DIJK, 1981).

FLOWERS et al. (1977) as well as STEWART (1971, 1972) interpret proline accumulation as the compensation of the osmosis potential, and with the fact that proline is the only amino acid which does not inhibit the activity of the enzymes even in a rather high concentration.

According to GÖRING and THIEN (1979) the increase in the proline concentration of leaves produces the longer staying of proteins in solution and strores such reducing energy which after the ceasing of waters tress appears in the form of NADH-H^+ during the course of the re-development of proline into glutamic acid.

PÁLFI et al. (1975) studied the proline accumulation characteristics of 46 soft-stalked, mainly cultivated plant species belonging to 14 plant families — their studies being related to the degree of water deficiency. Authors determined that on the effect of the same highleveled, lethal water deficiency even the species belonging to one family synthetize and accumulate completely differing amounts of proline. On the effect of water deficiency, therefore, the degree of proline accumulation is firstly a characteristic of species. Thus, the level of proline content and the degree of drought-resistance are only correlated in the case of cultivated types belonging to the same species, and in the case of subspecies and varieties, respectively.

PINTÉR et al. (1978, 1979, 1981), PÁLFI (1969), PÁLFI and JUHÁSZ (1971), PÁLFI et al. (1973, 1978), PÁLFI and PINTÉR (1980) studied the drought-resistance of paprika, sunflower, maize, lupine and rye with the help of the proline test. They studied 36 inbred corn lines and 12 hybrid corns. The inbred line having the highest proline concentration was crossed with two related corn lines and thus, an artificial population; then the "S_0" and "S_1" generations of this were established. The aim of the present study is to examine the drought-resistance of the two generations of the artificial population with the help of live-wilting and proline test, resp. By this means it can also be cleared whether the degree of drought-resistance of the inbred lines can be enhanced with the new method, that is, with crossing and selection on the basis of proline concentration.

Materials and methods

From the studied 36 inbred corn lines the "Le 60" line gave the highest proline concentration — 6.25 mg in 1 g dry-matter — on the effect of lethal water deficiency. "Three way cross", i.e. "TC" with (Le 60 x Le 24) x Le 28 was carried out with this line. The latter two lines are related to the "Le 60", having similar agronomical characteristics to it, and and weaker in respect of drought-resistance and the degree of proline accumulation. The proline accumulation caused by lethal water deficiency was 2.10 mg in the case of "Le 24" and 2.46 mg in the case of "Le 28" in 1 g dry-matter.

As in the previous experiments (PÁLFI et al. 1978; PÁLFI and PINTÉR, 1980; PINTÉR et al. 1978, 1979, 1981), for live-wilting the first leaf above the completely developed carpellary inflorescence was cut form each plant in the present study, too. From the "S_0" generation of the above-mentioned three way crossing, one leaf from each of a total of 64 plant individuals was cut off (taking a group of 16 plants four times according to the time of flowering). Using the method of live-wilting sublethal, then lethal water deficiency was produced gradually within 3 days — under constant light (5000 lx), at 24 C, in the isolated leaf samples. The moisture of the substance of the leaves exposed with their reverse sides upwards and fixed with transparent scotch tape (Fig. 1) was regulated, so that the water loss of the leaves was 20—25% within 24 h, 45—50% within 48 h (sublethal), and 65—75% within 72 h (lethal). By this means the "internal water deficiency" of each leaf after the three days was completely equal, that is, of lethal level. Then the leaves were separately cut into little dried pieces within 4—5 h, at 80°C. (air-dried material) and ground to dust. The proline determinations of the amino acid extracts were carried out with spectrophotometry according to the method of TROLL

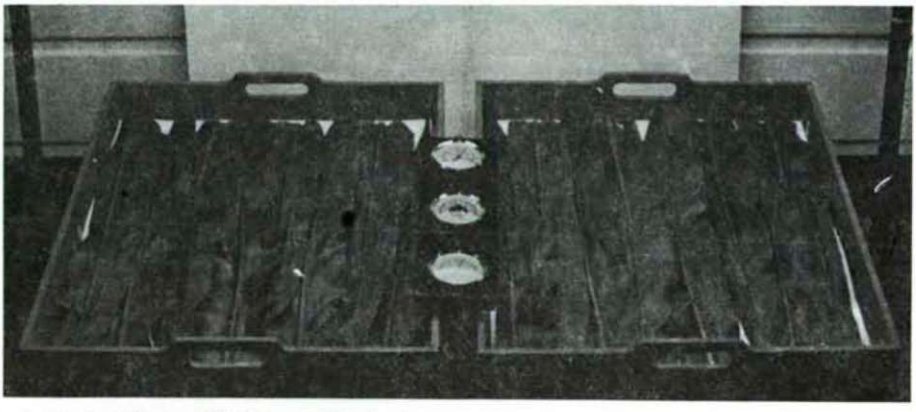

Fig. 1. The lethal water deficiency of the isolated corn leaves was gradually produced by the method of live-wilting. The leaves were placed in the trays close to each other so that they were situated with their shoulders on the opposite sides alternately, and with their reverse sides being upwards. The photosynthesis was functioned by illumination. The moisture of the air was daily set to 90, 80, and 60% during the three days. On the third day the lethal water deficiency, i.e. the equal level of "internal water deficiency" was observable in the case of every studied leaf.

and LINDSLEY (1955). The method of CHINARD (1952) was also applied for controlling. The demonstration of free proline was repeated four times, and if any of the repetitions varied from the average result by ±3% the analysis of the whole group of 16 plants was repeated.

From the 64 plant individuals of the "S_0" generation studied by leaf-analysis, crops of those 6 plants were separated where the cut off one-one leaf showed the highest proline concentration on the effect of lethal water deficiency provoked by live-wilting.

In the next vegetation year only the seeds of these 6 crops were sowed, and the plants developping from the seeds formed the "S_1" generation.

At the time of complete development of the carpellary inflorescence of the "S_1" generation plants 1—1 leaf specimen was taken again from 64 plant individuals and after the development of lethal water deficiency provoked by live-wilting the proline measurements were carried out again on the dried and crushed material. Then the results obtained in the case of the "S_0" generation were compared with those of "S_1".

Apart from the proline determinations, the quality analysis of certain amino acids and the measurements of the free protein-building total amino acids were also performed. The applied methods have already been reported earlier (PÁLFI and JUHÁSZ, 1971; PÁLFI et al. 1973, 1978; PÁLFI and PINTÉR, 1980).

Results and discussion

It has been determined that no quality changes appeared in the isolated leaves of the 64 plants of the artificial corn population on the effect of strong water deficiency in regard of the free amino acids. However, significant differences were demonstrated in the total amino acid concentration of the live-wilted leaves. Nevertheless, it became evident that the differences did not show relationship with the degree of proline accumulation.

From the amino acids and their amides the highest values were given by the concentration of asparagine and not proline. Furthermore, the amount of glutamine was also significant and was in many cases found to be accumulated in a higher amount than proline. Taking into consideration that no correlation was found between the degree of concentration of proline and other amino acids, and amides, resp. only the proline amounts are reported in relation to drought-resistance (Table 1).

Adding separately the proline concentration of the leaves of the 64 corn individuals according to the two generations (first and second year), it can be seen from the Table 1. that a significantly larger amount was obtained in the case of the „S_1" generation than in the „S_0".

Taking the average of the amounts yearly, according to the 64 plants, a proline concentration of 2.82 mg in the case of the „S_0" generation, and of 3.21 mg in the case of the „S_1" generation was demonstrable.

Taking the proline average of the „S_0" generation as 100%, the growth of the „S_1" generation was 13.83%.

It could be determined therefore that the proline concentration and degree of drought-resistance, resp. could be enhanced in the isolated leaves of the studied artificial corn population plants, by the means of selection according to the proline concentration caused by strong (lethal) water deficiency.

It was worthwhile of studying that, as a matter of fact, what significant changes took place between the plants of the „S_0" and „S_1" generations shown in Table 1, as the consequence of the selection carried out on the basis of the proline concentrations (on the effect of the provoked strong water deficiency). For comparison the proline concentrations of the 64 corn plants were divided into qualification categories per mg. The intervals between the highest and lowest proline concentrations received in the 36 inbred corn lines studied by us so far were taken as a base in forming the categories, independently of whether individuals occurred or not in the different categories in our present experiment (PÁLFI et al. 1978, 1980; PINTÉR et al. 1978, 1979).

No.	Proline concentration, in mg/1 g dry-matter		No.	Proline concentration, in mg/1 g dry-matter	
	S_0	S_1		S_0	S_1
1.	2.50	3.42	33.	2.59	2.08
2.	2.25	1.47	34.	3.05	2.53
3.	1.91	3.24	35.	2.81	2.57
4.	3.50	1.93	36.	2.54	2.83
5.	4.19	2.29	37.	2.45	1.96
6.	3.35	2.34	38.	2.59	5.58
7.	3.04	3.56	39.	4.14	2.10
8.	3.08	3.60	40.	2.95	3.14
9.	3.56	2.34	41.	4.24	2.21
10.	2.21	3.26	42.	4.12	2.35
11.	2.59	2.20	43.	2.84	2.89
12.	3.02	2.74	44.	2.12	2.66
13.	1.69	1.35	45.	4.03	2.43
14.	2.12	1.84	46.	2.75	1.89
15.	2.12	3.15	47.	2.18	5.10
16.	3.06	3.58	48.	3.07	4.12
17.	2.09	2.65	49.	2.70	5.10
18.	2.09	4.18	50.	4.64	5.24
19.	1.84	3.70	51.	3.56	1.75
20.	2.15	4.14	52.	3.53	2.65
21.	1.83	5.13	53.	3.51	4.10
22.	2.11	6.21	54.	2.93	2.16
23.	2.28	3.53	55.	3.15	3.38
24.	2.58	1.30	56.	1.94	2.12
25.	2.56	3.15	57.	2.54	2.10
26.	2.14	2.38	58.	5.63	6.88
27.	1.70	1.26	59.	2.97	4.45
28.	1.73	4.45	60.	2.59	5.16
29.	2.46	4.18	61.	2.61	4.59
30.	2.75	2.47	62.	2.07	3.60
31.	3.58	3.10	63.	2.93	4.06
32.	3.63	3.10	64.	3.15	4.10
	81.71	97.24		98.92	107.88

From 1 to 64, total: $S_0 = 180.63$ $S_1 = 205.12$
Average of the 64 leaves: $S_0 = 2.82$ $S_1 = 3.21$

The proline growth average of the generation compared: 13.83%

Table 1. Free proline concentration of leaves from 64 individuals of the "S_0" and "S_1" generations of the artificial population of the related, inbred corn lines, on the effect of strong (lethal) water deficiency.
From the individual plants No. 1 to 64 the number of proline mg-s totalized was 180.63 mg in case of "S_0"; and 205.12 mg in that of "S_1". From this the average of the 64 leaves was 2.82 mg in "S_0" and 3.21 mg in "S_1". The proline growth average of the "S_1". generation was 13.83% compared to the "S_0".

The category "rather high" appears three times between the 4 and 7 mg because in the case of our studies in the first year 4.3 mg was the highest proline concentration, and in the following years proline amounts above 5 and 6 mg were also obtained in the case of other lines. During the course of our experiments so far, however, no line has reached a proline level above 7.0 mg as yet.

The qualification category of rather low proline amount, i.e. under 1.0 mg can be seen in Table 2, since in the 36 inbred corn lines studied so far, such a low proline concentration has been observed in 4 lines.

It can be determined from Table 2 that the groups formed according to proline

Groups according to proline concentration, in mg/1 g dry matter	No. of individuals divided into groups		Qualification on the basis of proline amount
	S_0	S_1	
6 and 7 mg between	—	2	„Rather high"
5 and 6 mg between	1	6	„Rather high"
4 and 5 mg between	6	10	„Rather high"
3 and 4 mg between	16	15	„High"
2 and 3 mg between	34	22	„Moderate"
1 and 2 mg between	7	9	„Low"
0.5 and 1 mg between	—	—	„Rather low"
Total	64	64	individuals

Table 2. The isoated leaves of the 64 individuals of the "S_0" and "S_1" generations from the artificial corn population were divided into qualification categories on the basis of their proline concentration, what was provoked by the gradually developing lethal water deficienc of the leaves. Groups (categories) according to proline concentration in mg/l g dry matter. Numbe- of individuals divided into groups. Qualification according to the amount of proline: "Rare her high", "High", "Moderate", "Low", "Rather Low". A total of 64 individuals; betwetn 0.5—1 mg—1—2 mg—6—7 mg.

amounts show significant variations in the "rather high" category. 7 plants could be divided into this category in case of the "S_0" generation, and 18 in that of the "S_1". generation. The "S_0" and "S_1" generations did not show essential changes in the "high" and "low" categories. In contrast to this, there was a significant decrease in the amount of plants divided into the "moderate" category from the "S_1" generation, compared to the "S_0". Furthermore, the number of plants which could be divided into the "moderate" category in the case of the "S_1" generation (12) showed such a decrease, compared to which the number of plants from the same generation which could be divided into the "rather high" group showed an increase of almost the same amount (11). It can be concluded from this that the significant amount of plants from the "S_0" generation belonging to the „moderate" category immediately entered the

"rather high" group after proline selection; overstepping the group qualified as "high". Significant progress was obtained, therefore, by using the method of selection on the basis of proline.

VAN DE DIJK (1981) pointed out that it is not enough to produce an „external water deficiency" of the same level in the substance of the plant varieties to be studied, since this would cause various degrees of "internal water deficiency" in the plants having different grought-resistance. The consequence of this would be that due to the higher level of "internal water deficiency" of the less drought-resistant variety a higher amount of free proline would be synthetized and accumulated than in the case of the variety with higher drought-resistance (the internal water deficiency of which would be lower at this stage). A method was elaborated by the author with which the same level of "internal water deficiency" could be produced in the case of the plant variety to be studied.

With our live-wilting method (PÁLFI, 1969; PÁLFI and JUHÁSZ, 1971; PÁLFI et al. 1973, 1978; PÁLFI and PINTÉR, 1980; PINTÉR et al. 1978, 1979) the water deficiency of the isolated leaves developed gradually, reaching the sublethal state within 2 days, and by the third day the leaves of each studied plant reached the lethal level, by which time the „internal water deficiency" had no physiological significance any more.

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Address of the authors:
DR. G. PÁLFI
Department of Plant Physiology,
A. József University
H-6701 Szeged, P.O. Box 654,
ZSÓFIA PÁLFI
Institute of Plant Physiology
Biological Research Center,
Hung. Acad. Sci.
H-6701 Szeged, P.O. Box 521
DR. L. PINTÉR
Cereal Research Institute
H-6701 Szeged, P.O. Box 391
Hungary

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CHANGES IN THE AMOUNT OF DIFFUSIBLE AUXIN AND THE ACTIVITY OF IAA-OXIDASE FRACTIONS ON THE EFFECT OF 2-CHLOROETHYL-TRIMETHYL-AMMONIUM CHLORIDE (CCC)

MÁRIA NAGY and ZSUZSA TABI
Department of Plant Physiology, Attila József University, Szeged
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Key words: auxin, diffusion, permeability, IAA-oxidase, *Phaseolus vulgaris*

Abstract

In bean plants the content of diffusible IAA increased on the effect of a treatment with CCC, indicating that the auxin supply of the tissues in the elongation zone did not decrease on the effect of the retardant. The increase of the amount of diffusible IAA may be correlated with the effect of CCC on the permeability of the membrane. The increase of activity of the ionically wall-bound IAA-oxidase measurable on the effect of the treatment is playing a definite role in the limitation of elongation.

Introduction

The observations according to which the endogenous IAA content of the plants decreased on the effect of a CCC-treatment (KURAISHI and MUIR, 1963; NORRIS, 1966; VOLYNETZ and PALCHENKO, 1977) fitted well into the concept developed for the mechanism of effect of the growth retardants.

However, in the regulation of growth in the first line not the total IAA content of the plants but rather the IAA-supply of the tissues present in the elongation zone is the factor of importance which is correlated also with the movement of auxin within the plant.

Informations concerning the latter cannot be obtained by the measurement of the total IAA content extractable from the homogenizate of the plants, particularly in case of a treatment with compounds such as CCC which causes a significant change in the permeability of the tissues (FABIJAN et al., 1981) and thus presumably also in the movement of auxin.

Materials and methods

For our investigations we used bean cultivars of various growth intensities (*Phaseolus vulgaris convar. vulgaris cv. Juliska and convar. nanus cv. Cherokee*). The seeds were swollen in a CCC (Merck-Schuchardt) solution of 1000 mg/l concentration in a 25 °C thermostat, then sown in garden mould and grown under controlled conditions.

The content of diffusible auxin was determined in the shoots above the cotyledone of plants aged 9 days by diffusion accelerated by centrifugation as modified according to the method of GOLDSCMIDT and MONSELISE (1968). The excised shoots were covered in a vertical position with 20% methanol and centrifuged 45 minutes at 1500 g. The diffusate was evaporated under reduced pressure to an aqueous residue and a 0.5 M solution of K_2HPO_4 was added to it (pH 8.5). The purification and fractionation were carried out according to the method of KNEGT and BRUINSMA (1973), further of HEMBERG and TILLBERG (1980). The indole compounds present in the final ethereal fraction were separated by TLC, using a solvent system of chloroform: ethyl acetate: formic acid (4:5:1). The amount of IAA was determined with a SPEKORD UV/VIS photometer, on applying the method of (FLETSCHER and ZALIK (1964).

IAA-oxidase preparation: 10 g epicotyl was ground with a double amount of phosphate buffer pH 7.2) and centrifuged 5 minutes at 1000 g. The supernatant was again centrifuged 20 minutes at 20 000 g. The supernatant obtained in this way represented the soluble fraction and the residue the membrane fraction (DARIMONT et al., 1977). The residue obtained at the first centrifugation was incubated 12 hours with a 0.3 M NaCl solution (SÁGI, 1979) and subsequently centrifuged 15 minutes at 12 000 g. The supernatant contains the ionically bound wall-peroxidases. The covalently bound fraction was obtained by the method of DARIMONT et al., (1973), by the treatment with pectinase (SIGMA) and cellulase (MERCK).

Polyclar-AT was used at the preparations. The protein content was measured according to the method of LOWRY et al., (1951).

IAA-oxidase assay: the amount of decomposed IAA was determined by photometry, using the method of GALSTON and DALBERG (1954), further of HILLMANN and GALSTON (1956). The reaction mixtures contained besides the enzyme extract and IAA also $MnCl_2$, DCP and H_2O_2. The samples were incubated 60 minutes at 30°C then 2 ml of GORDON-WEBER reagent was added to 1 ml of the sample and the developed colour determined after 30 minutes by photometry at 530 nm.

Results and discussion

On the effect a treatment with CCC the amount of diffusible auxin increased in comparison to those of the controls, both in case of normal and of dwarf beans (Table 1). The results indicate that the decrease of the extractable auxin content

Table 1. Effect of CCC on the amount of diffusible IAA

	diffusible IAA ng/ 100 plant	
	control	treated
cv. Juliska	40	49
cv. Cherokee	31	43

(KURAISHI and MUIR, 1963; VOLYNETZ and PALCHENKO, 1977) does not mean at the same time a weaker supply of auxin of the tissues present in the elongation zone, and they are calling our attention to the fact that owing to the increase of membrane permeability (FABIJAN et al., 1981) the auxin supply in the treated plants may be even better than that of the controls.

The effect of the treatment with CCC on the fractions of IAA-oxidase is shown by the data of Table 2. An increase of activity was experienced in the soluble and in the ionically wall-bound fractions.

Table 2. Effect of CCC on the fractions of IAA-oxidase

		IAA destroyed ($\mu g . mg^{-1}$ protein h^{-1})			
		soluble	membrane	wall-bound	
				ionic	covalent
cv. Juliska	control	12	13	22	12
	treated	16	14	38	12
cv. Cherokee	control	13	12	25	16
	treated	17	13	31	15

The increase of the activity of cytoplasmatic IAA-oxidase on the effect of CCC has been indicated also by other authors (HALEVY, 1963; EL-FOULY and JUNG, 1965; GASPAR and LACOPPE, 1968). However, from the aspect of a more direct correlation between the extension growth and the IAA-oxidase, taking into account the changes in the ultrastructure of the cell wall, rather the change of the activity of IAA-oxidase bound ionically to the cell wall is more worthy of attention.

The activity of the ionically wall-bound IAA-oxidase was determined also in the non-growing epicotyls and it was found that in the non-growing epicotyls of untreated plants the activity of this IAA-oxidase fraction was higher than in the growing epicotyl (destroyed IAA 30 $\mu g \cdot mg^{-1}$ protein $\cdot h^{-1}$). Therefore we presume that the ionically wall-bound IAA-oxidase is playing a definite role instead of the control of the rate of elongation, rather in its limitation and its conversion to irreversibility. Similar results were obtained by SÁGI (1979) in the case of lupin. The IAA-oxidase of the cell wall participates in the synthesis of lignin (STAFFORD, 1965; HARKIN and OBST, 1973) whereas the accumulation of lignin may be one of the causes of the discontinuance of growth WHITMORE, 1971). Thus, in the development of the retarding effect of CCC a significance must be ascribed also to the increased lignin synthesis.

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Adress of the authors:
DR. MÁRIA NAGY
ZSUZSA TABI
Department of Plant Physiology
A. J. University, H-7801 Szeged
P.O. Box 654, Hungary

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EFFECT OF NITROUS OXIDE ON PLANT CELL DIVISION: THE CYTOLOGICAL SIDE-EFFECT OF N_2O TREATMENT

ANNA SZELES

Department of Anthropology, Attila József University Szeged
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Abstract

Chromosome aberrations and disturbances of cell division in plant meristematic cells caused by long term nitrous oxide treatment are described. On the basis of detected aberrations, e.g. aneuploidy, polyploidy, irregularities of chromatin condensation, and micronucleus formation, the mutagenic activity of nitrous oxide is very probable. Since nitrous oxide is widely used in clinical practice the full-range mutagenicity test of nitrous oxide would be essential.
Key words: rye, nitrous oxide, chromosome aberrations, mitotic defects, micronuclei, mutagenicity.

Introduction

In a recent paper (SZELES, 1982) I have described the effect of nitrous oxide on mitosis of rye root-tip meristem cells. The metaphase blocking effect of N_2O was found to be reversible and following the termination of gas treatment the metaphase cells proceeded to more or less normal anaphases. Numerous aberrations of chromosomes and cell division were also observed which could be regarded as the cytological side-effects of N_2O treatment. Since, the nitrous oxide is widely used in clinical practice, the study of cytological disturbances caused by N_2O treatment seems to be necessary.

In the present paper chromosome aberrations and disturbances of cell division caused by long term nitrous oxide treatment are described. Instead of a complete registration and presentation of cytological findings only the most important groups of aberrations are reported: 1. micronucleus formation, 2. alterations in chromosome number, and 3. irregularity in condensation of chromosomes and nuclei.

Materials and Methods

Nitrous oxide (N_2O) treatment of rye (*Secale cereale*, $2n=14$) seedlings and cytological investigations were carried out as described previously (SZELES, 1982). Cytological examinations and microphotographs were made with a ZEISS NU-2 light microscope.

Results and Discussion

Cytological disturbances of plant meristematic cells treated with nitrous oxide were grouped into three categories: 1. micronucleus formations, 2. alterations in chromosome number, and 3. irregularities of chromatin condensation. These categories are in close connection in respect of their evolution and manifestation.

1) **Micronucleus formation**

The most characteristic cytological side-effect of nitrous oxide treatments was found to be the formation of micronuclei. The first appearance of micronuclei was detected after 4 a hour treatment at 6 atm pressure (see SZELES, 1982), and the number

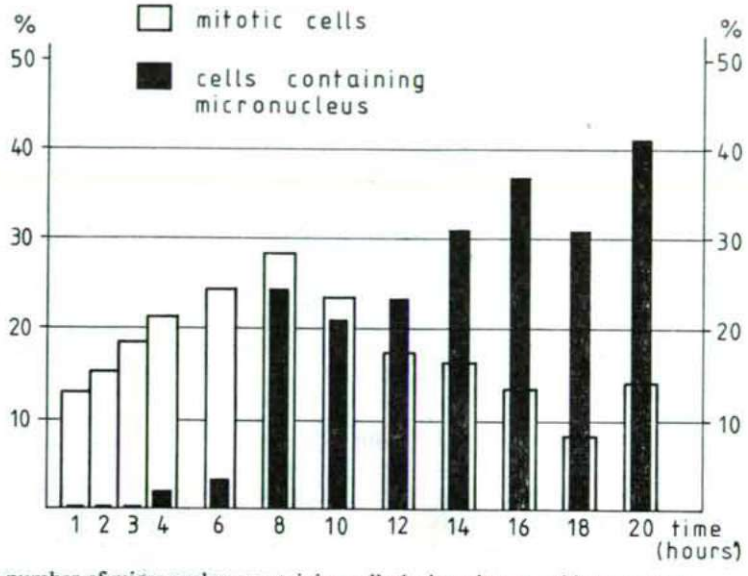

Fig. 1. The number of micronucleus containing cells during nitrous oxide treatment.

of micronuclei was dramatically increased at 6—8 h treatment. From 12 h treatment only a gradual increase of number of micronuclei was found (Fig. 1), showing that the disintegration of chromosomes starts approx. at the 10th hour of the gas treatment. Presence of micronuclei was also noticed after very short (2 hour) treatment. Obviously, these micronuclei were generated from the cells which were in mitosis at the beginning of the gas treatment.

The first cytological mark of the micronucleus formation was the separation of individual chromosomes or groups of chromosomes in metaphase (Fig. 2, picture 1—4). These separated chromosomes preserving their integrity were gradually decondensed lacking the normal anaphase process and went to telophase (Fig. 2, pictures 5—12). In telophase, decondensation of chromosomes showed high degree of synchrony, regardless of the localization of chromosomes or chromosome groups within the cells (Fig. 3, pictures 1—6), and parallel with the formation of interphase nuclei the formation of micronuclei was proceeded (Fig. 3, pictures 7—12). During the N_2O treatment not only the number of cells containing micronucleus was increased (Fig. 1) but an increase in the number of micronuclei per cell was also found (Fig. 3, pictures 7 and 11). Micronuclei were very often associated (Fig. 3, picture 8) and overlapped on each other (Fig. 3, picture 11), therefore, the statistical analysis of the number of micronuclei per cells proved to be inadequate.

The mutagenic and micronucleus inducing activity of certain chemical agents shows direct correlation. In the last decade, using that correlations, the „micronuc-

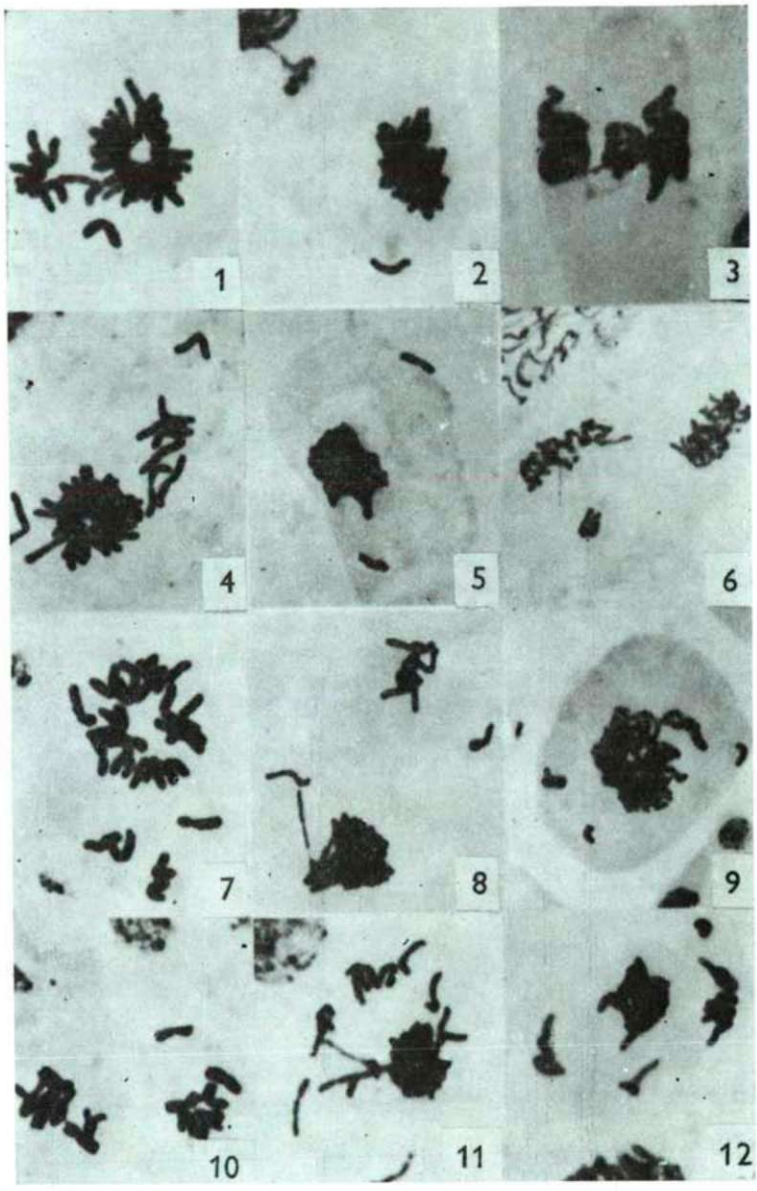

Fig. 2. Process of micronucleus formation (Magnification 650x)
1, 4, 6, 7, and 10 (6 atm 8 hours)
2, 3, 5 and 8 (6 atm 12 hours)
11 and 12 (6 atm 2 hours)
9 (6 atm 26 hours)

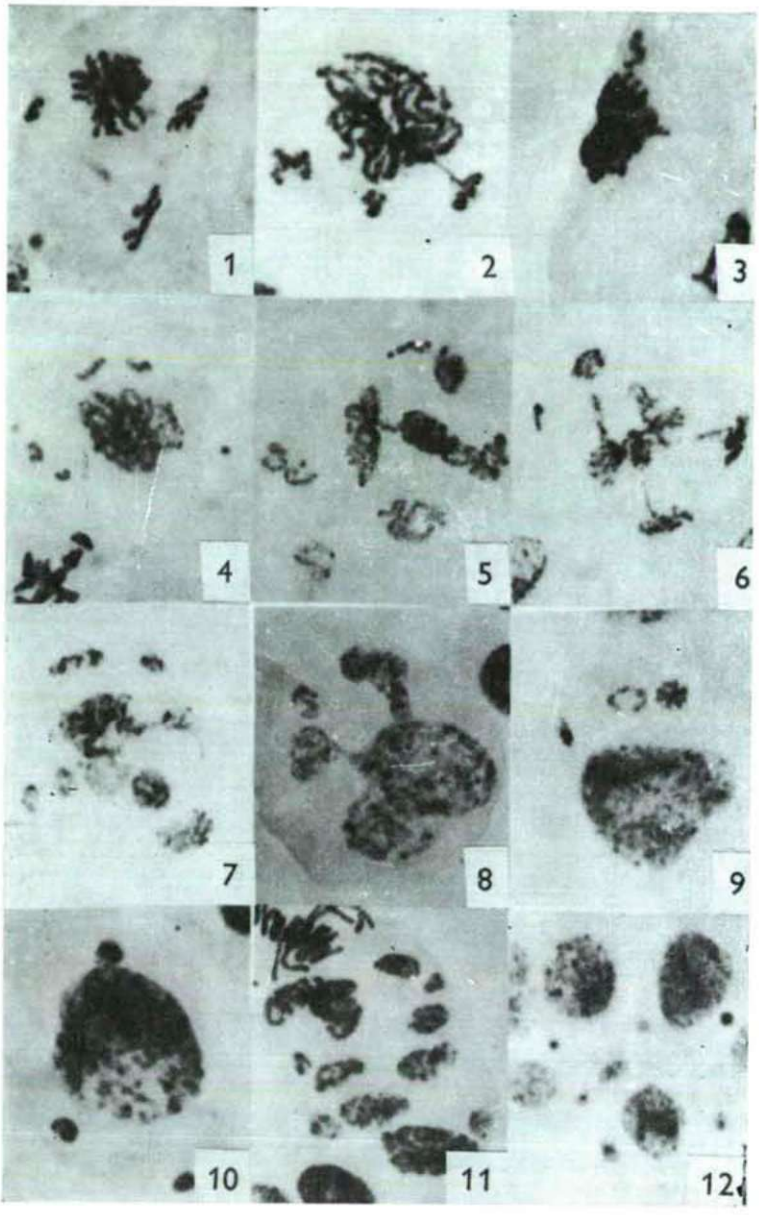

Fig. 3. process of micronucleus formation (650x)
1,9 and 12 (6 atm 8 hours + 30 min)
2,3 and 4 (6 atm 12 hours)
5 (11 atm 2 hours)
6 (12 atm 2 hours)
7 (7 atm 2 hours)
8 and 11 (9 atm 2 hours)

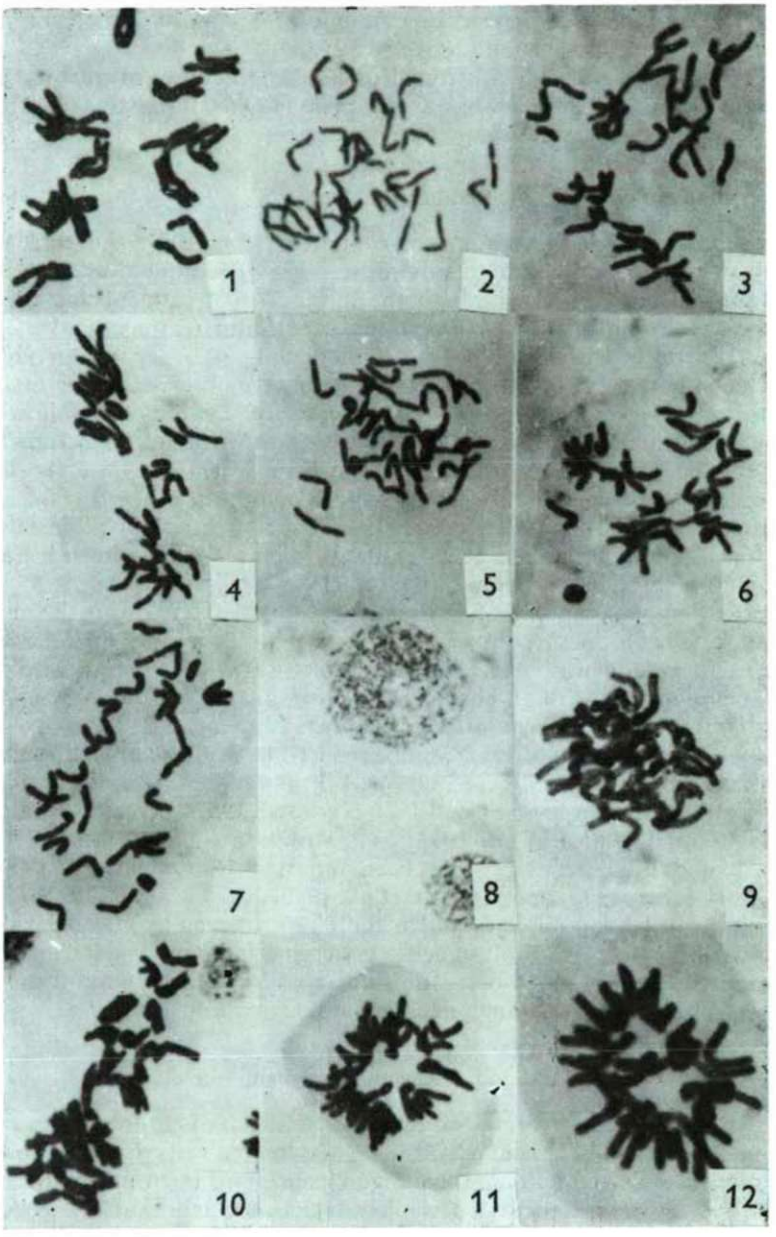

Fig. 4. Formation of polyploid cells by N_2O treatment (800x)
1 and 6 (6 atm 8 hours)
2 (12 atm 2 hours)
3, 4, 7, 10, 11 and 12 (6 atm 12 hours)
5 (11 atm 2 hours)
8 (6 atm 8 hours + 5 hours)
9 (6 atm 8 hours + 10 hours)

leus test" of the eukaryotic oragnism became one of the most important methods in the mutagenic tests (BOLLER and SCHMID, 1977).

Considering the significant micronucleus inducing activity of nitrous oxide, in respect to the common utilization of nitrous oxide in clinical practice, the full-range mutagenic test of nitrous oxide seems to be essential.

2) Alterations in chromosome number

In the alterations of the chromosome numbers caused by the nitrous oxide the most pronounced was the polyploidy. Formation of tetraploid and higher polyploid cells showed correlation with both the duration of treatment and the applied pressure of gas. Tetraploid cells have been found at 6h 6 atm treatment and at 2 h 9 atm treatments. Disarray of chromosomes (Fig. 4, pictures 1—4) or separation of chromatids in the metaphase ring (Fig. 4, pictures 11—12) were observed as the first cytological marks of polyploidization. In the absence of cytokinesis, following the replication of chromosomes tetraploid cells were formed (Fig. 4, pictures 8—12). Increasing the time of the N_2O treatment (to 20 hours at 6 atm) the repetition of aforsaid process, higher polyploid (octoploid) cells could be formed (Fig. 5, pictures 1—4). Irregularity in this process might cause an assimetric polyploidization and results in formation of hexaploid cells. Figure 5 (picture 5) shows such a hexaploid cell that contains three independent diploid nuclei.

The formation of aneuploid cells by means of citokinesis following the micronucleus formation (Fig. 6, pictures 1—2) and (or that following polyploidization and micronucleus formation was detected (Fig. 6, pictures 3—4). Figure 6 (picture 5) shows an aneuploid cell with 16 chromosomes and picture 6 shows cell containing only 3 chromosomes and a chromosome fragment.

The alterations in chromosome number caused by N_2O treatment are in agreement with data reported by different authors. Nitrous oxide induced polyploidy has been described by ÖSTERGREN, 1954, 1957; NYGREN, 1955; KIHARA et al., 1960; TSUNEWAKI, 1962; DVORAK et al., 1973; SUBRAHMANYAM and KASHA, 1975. Also, nitrous oxide induced aneuploidy has been reported by ÖSTERGREN, 1954, 1957; DVORAK and HARVEY, 1973; and DVORAK et al., 1973.

It is noteworthy that polyploids and aneuploids produced by nitrous oxide treatment might serve as potential tools in cell genetics and in plant genetics.

The results of the present observations strongly suggest that the formation of aneuploid cells is carried aut through micronucleus formation.

3) Irregularities in condensation of chromosomes and nuclei

Different irregularities of organization (condensation) of chromosomes and nuclei were found to be characteristic to nitrous oxide treated cells. Figure 7 (picture 1) shows an interphase cell containing three highly condensed micronuclei. In the cells, in which the nucleus was fragmentated by micronucleus formation, different degree of condensation of micronuclei was often detected. Figure 7 (pictures 2—7) shows cells

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Fig. 6. Formation of aneuploid cells
1, 2, 3 and 6 (6 atm 20 hours)
4 (6 atm 8 hours)
5 (6 atm 8+15 hours)
Magnifications: 1, 2, 4 and 5=800x
3=600x
6=730x

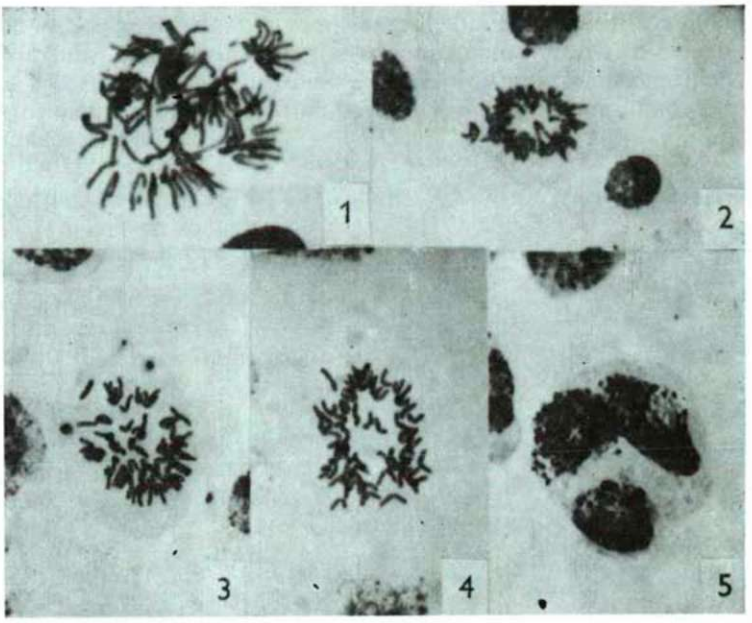
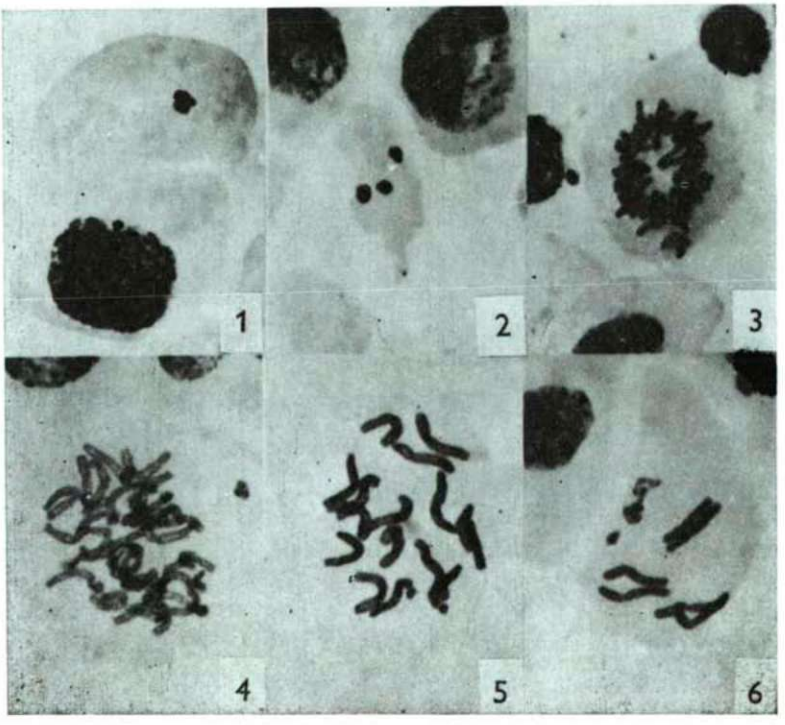


Fig. 5. Poliploid cells produced by N_2O treatment (730x)
1 (6 atm 8+15 hours)
2 and 3 (6 atm 20 hours)
4 (6 atm 26 hours)
5 (6 atm 24 hours)



containing more or less condensed chromosomes and interphase micronuclei as well. Different degree of chromosome condensation was also found, and prophase and metaphase chromosomes were visible in the same cell (Fig. 7, picture 7—9) or among metaphase chromosomes highly despiralized chromosomes were also found (Fig. 7,

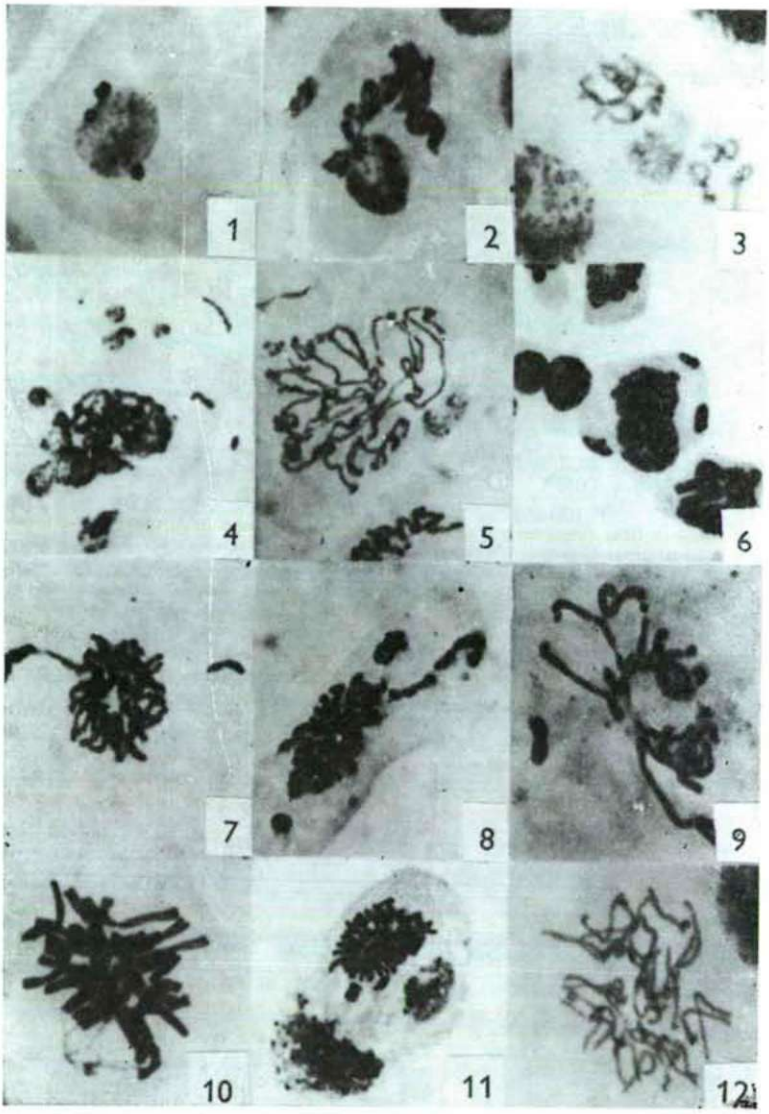


Fig. 7. Irregularities of chromatin condensation (800x)
1 and 4 (6 atm 20 hours)
2, 5, 8 and 9 (6 atm 8 hours)
3 (6 atm 8+5 hours
6 (6 atm 26 hours)
7, 10 and 11 (6 atm 12 hours)
12 (6 atm 8 hours+30 min)

picture 10). Figure 7 (picture 11) represents an assimetric polyploid cell containing both interphase and mitotic nuclei. In some cases irregular centromere division and chromatid separation was detected. On figure 7 (picture 12) a cell is shown in which

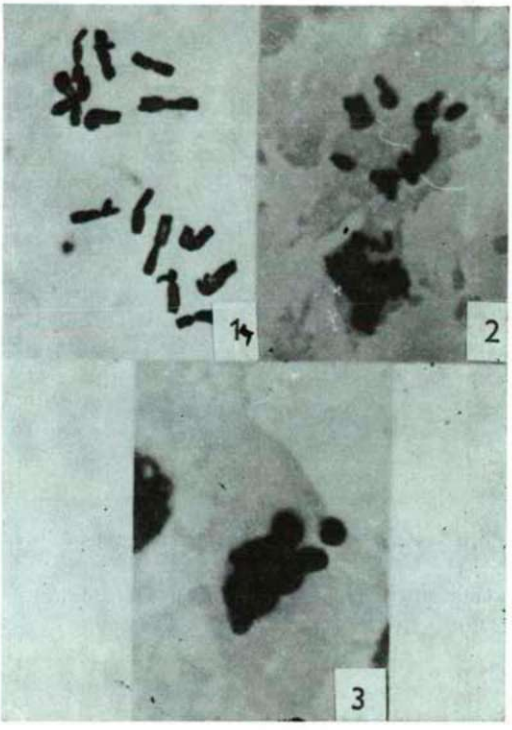


Fig. 8. Chromosome breakage (picture 1, arrow) and disintegrated nuclei or chromosomes after 6 atm 8 hours treatment (pictures 2 and 3)

the separation of chromatids and the division of centromeres are accomplished at prophase.

There is an open question whether the irregularity of chromatin condensation is a specific effect of the nitrous oxide or simply the physical consequence of the hight pressure.

It should be noted that in the early hours of the treatment, at high nitrous oxide concentrations, breakage of chromosomes (Fig. 8, picture 1) disintegrated presumably-mitotic cells (Fig. 8, pictures 2—3) were observed too. After termination of gas treatment the most frequent anaphase abnormalities were the unequal chromosome distributions (Fig. 9, pictures 1—4), anaphase bridges (Fig. 9, pictures 4—5), and chromosome separation (Fig. 9, pictures 3—6). These irregularities might also play an important role in the aneuploid formation.

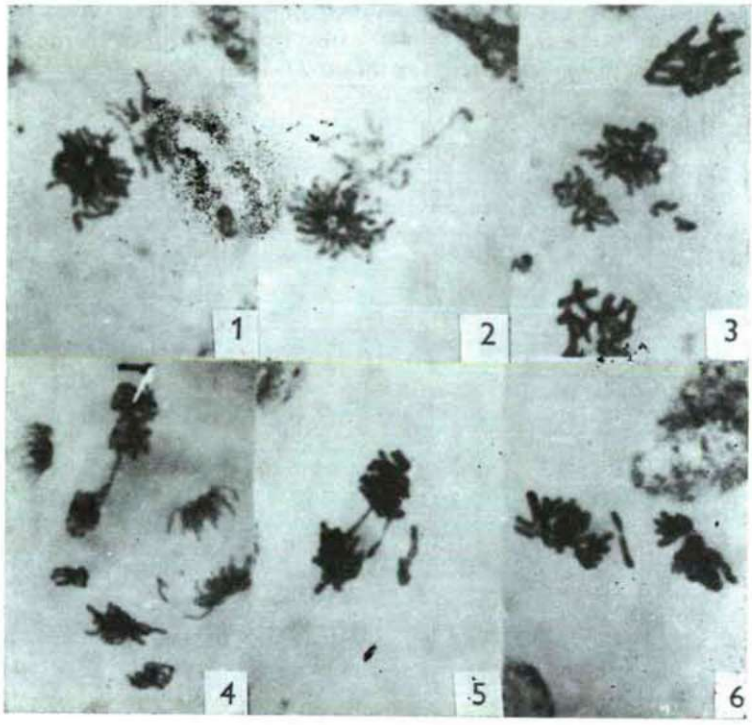


Fig. 9. Anaphase abnormalities after terminating a 6 atm nitrous oxide treatment. Magnification: 730x

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Address of the author:
DR. ANNA SZELES
Department of Anthropology,
Attila József University,
H-6701 Szeged, P.O. Box 660
Hungary

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EFFECT OF SHORT PERIODS OF LIGHT ON THE ORGANIZATION OF THE MEMBRANEOUS SYSTEM OF CORN MESOPHYLL CHLOROPLASTS

I. MARÓTI and EDIT TAKÁCS

Department of Botany, Attila József University, Szeged
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Abstract

The effect of 16—8 h and 15—7.5 min light-dark cycles (LDC) on the lamella system of mesophyll chloroplast (Mchlp) was studied in the fourth leaves of five weeks old corn plants grown in phytotron. In the long and short LDC-s the period of light was the same (16 h/day), the light intensity was 32 Wm^{-2}.

Compared to the 16—8 h control, the 15—7.5 min LCD showed the following effects:

— The cut-surface quota (area) of the grana almost decreased to the half;
— In the case of corns 523 broader grana of lower height developed. The grana made up of 2—4 thylakoids were predominating (cc. 50%), while those standing of 14—35 thylakoids were missing;
— In the case of corns 165 and 3901 the thylakoids of the high grana became swollen, the partitions became loose, "decomposed". Stroma lamellae were frequent in these M chloroplasts.
— In the Mchlp starch occurred in lower amount and smaller size.

Introduction

It was determined by BLACKMAN in 1905 that photosynthesis has two limiting factors: photochemical reactions in case of low light intensity, and dark reactions in that of high light intensity.

The significance of the length of light periods on the development of plants and the usefulness of light was later confirmed by several authors: WARBURG (1919); GARNER and ALLARD (1931), EMERSON and ARNOLD (1932), GREGORY and PEARSE (1937), PORTSMOUTH (1937), BONDE (1955), HILLMAN (1956), WHITTINGHAM and BROWN (1953), FOGG (1968), POLLARD (1970), RAJAN et al. (1971), HORVÁTH et al. (1977, 1978) and others.

KETELLAPER (1964) holds the ratio of photoperiod and whole cycle length as an important factor. Besides the daily illumination and length of photoperiod, great importance is also attached to the length of dark periods in the short periods of light (MARÓTI et al. 1981; MARÓTI and PATAKY, 1982; MARÓTI, 1982).

It is known that the various lengths of light-dark periods have different effects on the growth of plants, however, there are only few data on its relationship to the internal membraneous system of chloroplasts.

It is assumed that the length and ratio of the light-dark period basically determine the organization of the chloroplast membraneous system. On the basis of our earlier studies (MARÓTI and GÁBOR, 1976; MARÓTI, 1982) it may also be presumed that the area and ratio of the single and stacked lamellae also have important role in the light utilization of certain plants.

It is not clear as yet in what degree the light and genotype, resp. determine the ratio of the single and stacked membranes. In fact, even the relationships between the

light intensity and granum-formation are evaluated diversely (BOARDMAN et al. 1974; VLASZOVA and OSZIPOVA, 1973).

For studies on the organization of the chloroplast membranes — STRASSER and BUTLER (1976), ARMOND et al. (1976), AKOYUNOGLOU et al. (1978) — such light periods are frequently applied, where the length varies from 0.01s to 2 min. These „flash" light experiments cannot be compared to our 15—7.5 min LDC-s, since here the light-dark ratio is 1/50 or less, and on the other hand no normal grana formation can be observed in adhered double, so-called primary thylakoids.

Compared to the 16—8h long illumination the 15—7.5 min light-dark cycle (LDC) significantly descreased the Chl a/b protein complex II. pigments on the one hand (MARÓTI, 1982), and in a degree depending on the genotype it shrinks and flattens the corn mesophyll chloroplasts on the other hand (MARÓTI and PATAKY, 1982).

The question arises whether the decrease in neoxanthin, lutein and Chl-b, and the flattening of the chloroplasts, resp. are in connection with the thylakoid number per granum.

In this paper a demonstration is given of the relationship between the afore-mentioned effects of the 15—7.5 min LDC and the shape, number and area-ratio of the grana.

Materials and methods

Zea mays L.: 165 and 523 Pioneer* inbred lines and 3901 hybrids were used for studies. The plants were grown in phytotron — HORVÁTH (1972) —, on a mixture of perlit and sand 1:1 (volume), HOAGLAND — REYSS et BOURDU (1970) — in nutriment solution with light intensity of 32 W/m² and constant temperature of 20 ± 1 °C.

The daily amount of light (light intensity x period) was the same. The control plants were grown in 16h continuous light and 8 h dark, while in the short cycle experiments alternating periods of 15 min light and 7.5 min dark were applied. Light tubes F_{29} served as the source of illumination.

The water capacity was 70%. The plants were watered daily with distilled water, the aliments were supplemented with 20 ml of nutriment-solution twice weekly. The dry weight of the five weeks old plants was measured according to the different organs after drying at 70 °C.

For electronmicroscopic studies samples were taken from the middle part of the fifth leaves. The leaf pieces with diameters of 0.5—1 mm were fixed in 3% glutaraldehyde, then contrasted with 2% KM_nO_4 solution, dehydrated in an ethanol series, and embedded into a Durcupan-ACM mixture. The sections prepared with a Reichert ultramicrotome were stained with Pb-citrate.

Photographs were prepared of the mesophyll chloroplasts with Tesla BS 242E and Tesla BS 50 electronmicroscopes. On the photograps of the given magnifications measurings were carried out on the planar size of the plasts (where the envelope was not observable well enough, the line of the stroma was taken as the limit of the area), the number, "width" and "height" measurements of the grana, as well as the amount of granum) thylakoid. 30—40 plasts were evaluated according to the various treatments.

Results

1. AREA-RATIO AND AMOUNT OF GRANA

In the 16—8 h LDC the area of grana amounted to cc 30% of the cut-surface (area) of the whole chloroplast in the three corn geno-types. In these chloroplasts 20—25 grana could be found per cut-surface. Regarding the number and area-ratio of granum no significant changes could be observed between the various corn genotypes.

The ratio of grana, as well as the single stroma membranes and the stroma, resp. cannot be reliably given here due to the inadequate resolution of the BS 242 E electronmicroscopic photographs.

*The corn kernels were obtained from Dr. LÁSZLÓ KÁLMÁN, Cereal Research Institute of Szeged.

In the 15—7.5 min LDC the surface quota of the grana (10—15%) decreased nearly to the half compared to the 16—18 h control. The granum number slightly decreased — 10—15 per cut surface — on the effect of the short light period. The lower decrease in the amount of grana and the higher decrease in the grana area was in connection with the fact that in the 15—7.5 min LDC the grana were made up of less thylakoid than in the 16—8 h LDC.

2. THE EFFECT OF SHORT LIGHT PERIOD ON THE SHAPE OF GRANA AND THE RATIO GRANA/THYLAKOID

During the period of continuous illumination (16—8 h LDC) grana consisting of many compartments developed by the multiple adherence of the thylakoids (Th) in the mesophyll chloroplasts (M chlp), in the case of all three genotypes of corns. To determine more exactly the grana/thylakoid ratio the grana were separated into groups, namely, those made up of 2—4, 5—8, 9—10, 11—12, 13—14, 15—20, 20—35 thylakoids. The distribution according to percentage was calculated in tese 7 groups (Fig. 1).

In all three types of corns the most frequently occurring grana were those consisting of 5—10 Th-s. Grana made up of the highest amount of Th and the lowest amount of 2—8 Th-s were found in the Mchp of the corn 165.

The number of grana consisting of 2—8 thylakoids was significant in the Mchp of the line 523, and in the hybrid corn 3901 the distribution of the low and high amount of thylakoid-containing grana was proportional.

It seemed a general rule that with the increase of thylakoid aggregation:

— the grana developed the form of narrow and high columns, their height/width ratio increased,
— the various partition lengths and the stacking surface of the various grana compartments decreased.

The 15—7.5 min LDC showed partly uniform and partly genotypdependent influence on the mesophyll chloroplast structure of the three corn types.

The general effect of the short cycle was that the intact grana consisting of 14—35 thylakoids did not develop, while those made up of 2—4 thylakoids occured in 50—70% (Fig. 1).

The differing effect of the 15—7.5 min LDC showed manifestation on the organization of the internal lamella system in two basic forms.

One of the types — represented by corn 523 — was characteristic of the fact that the ratio of the stacked and single lamellae did not show essential differences, nevertheless:

— such grana developed which lower height and were wider;
— the grana made up of 2—4 thylakoids were predominant (cc 50%), those standing of 5—8 Th-s were also frequent, (cc. 30%), and those having 14—35 Th-s were missing;
— there was an increase in the stacking surface of the grana disc, and in the ratio of the end granal membranes;
— the grana showed a more sporadical arrangement than in the 16—8 h LDC (Plate II, Fig. 1).

The other type of effect of the short cycle was observable in the Mchp-s of the corns 165 and 3901. Here the ratio of the length of grana and stroma lamellae showed significant alteration on the effect of the 15—7.5 min LDC, since:

— the membranes of the high grana showed "mosaic" swelling, became loose and decomposed;

— whole grana disappeared as the consequence of the destruction of the granum partitions, therefore the stroma lamellae predominated in these chloroplasts (Plate I, III).

3. Detection of starch in the mesophyll chloroplasts

Generally 40 M chloroplast sections were studied per corn. In the 16—8 h LDC the highest amount of starch was found in the hybrid 3901, 3—4 were frequent per plast. Starch was observed in about 50% of the M chloroplasts of corn 165. The

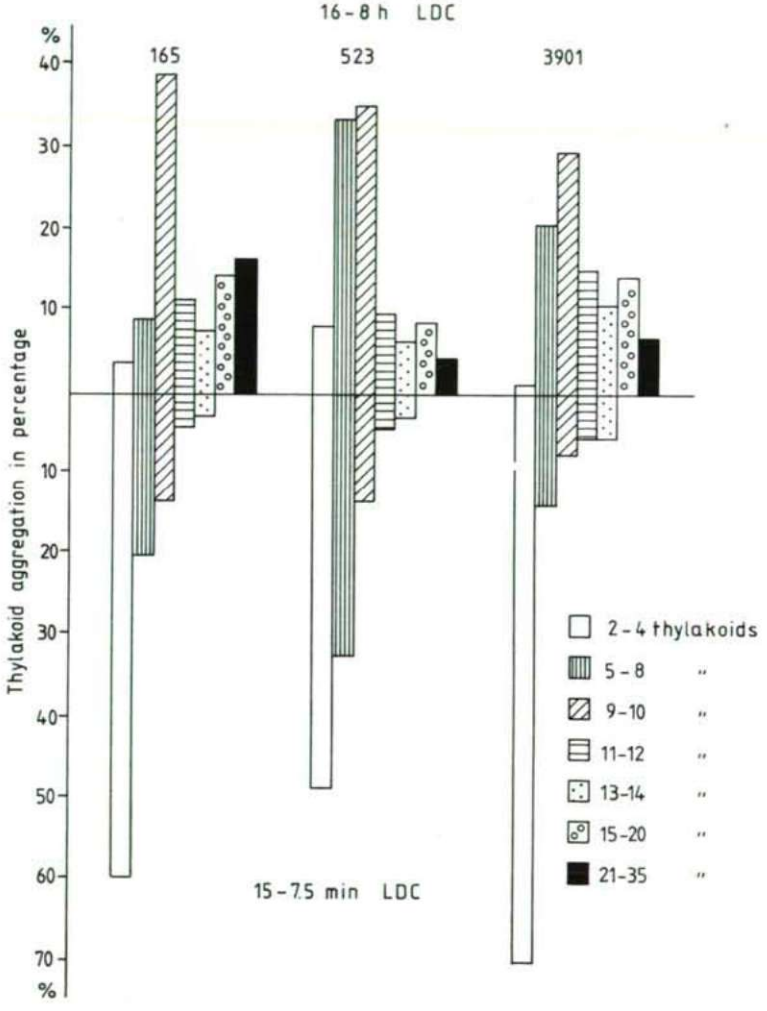
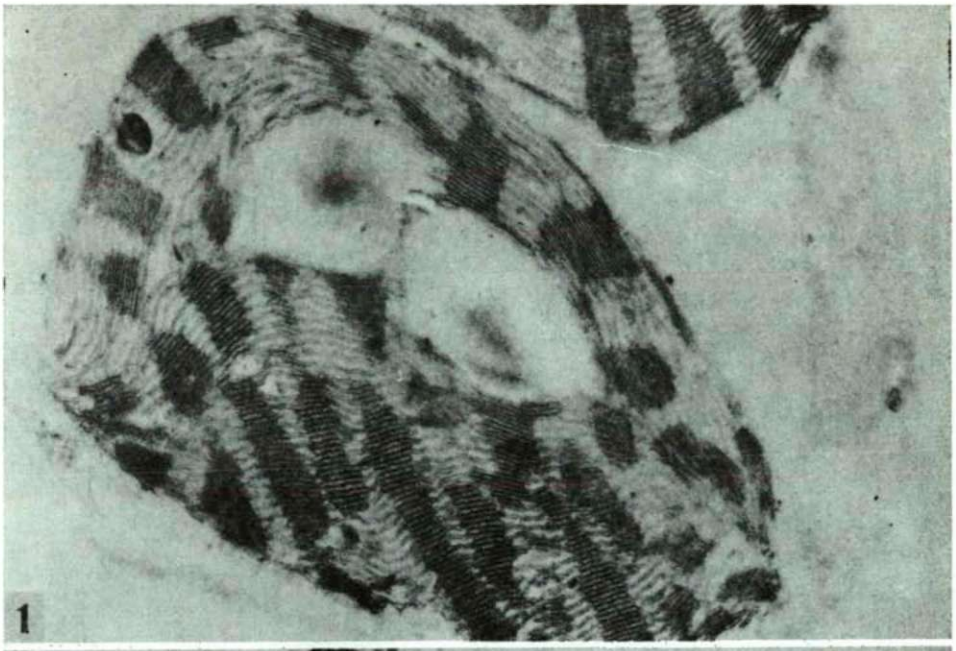


Fig. 1. The percental change of thylakoid aggregation on the effect of long (16—8 h) and short (15—7.5 min) light periods. The sample was taken from the three corn genotypes (P 165, P 523, P 3901) at five weeks of age, from the middle part of the fifth leaves.

Plate I. *Zea mays* L. *Pioneer* 165

1. light-dark cycle 16—8 h (30 000 X)
2. light-dark cycle 15—7.5 min (30 000 X)



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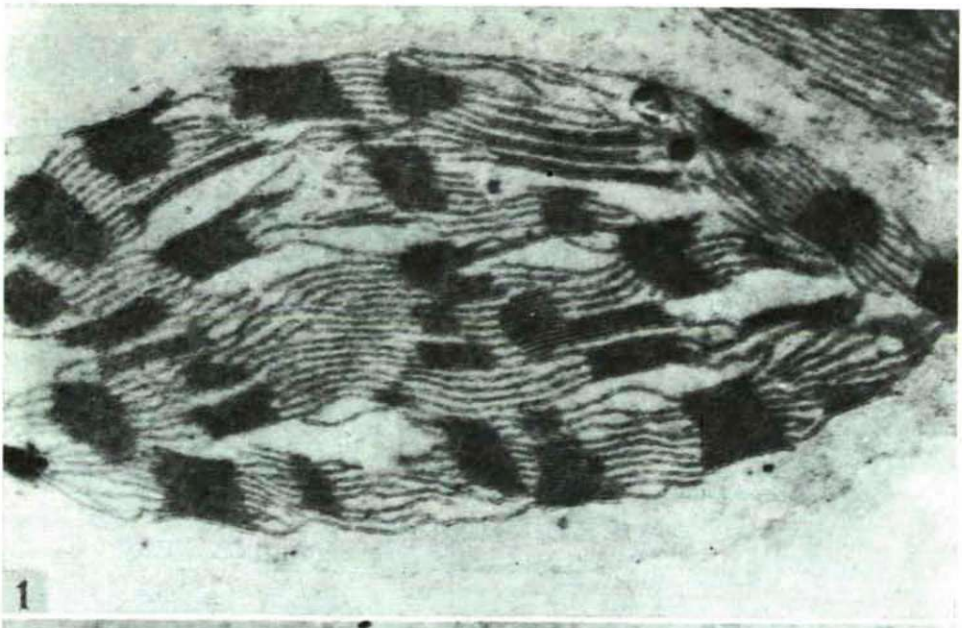

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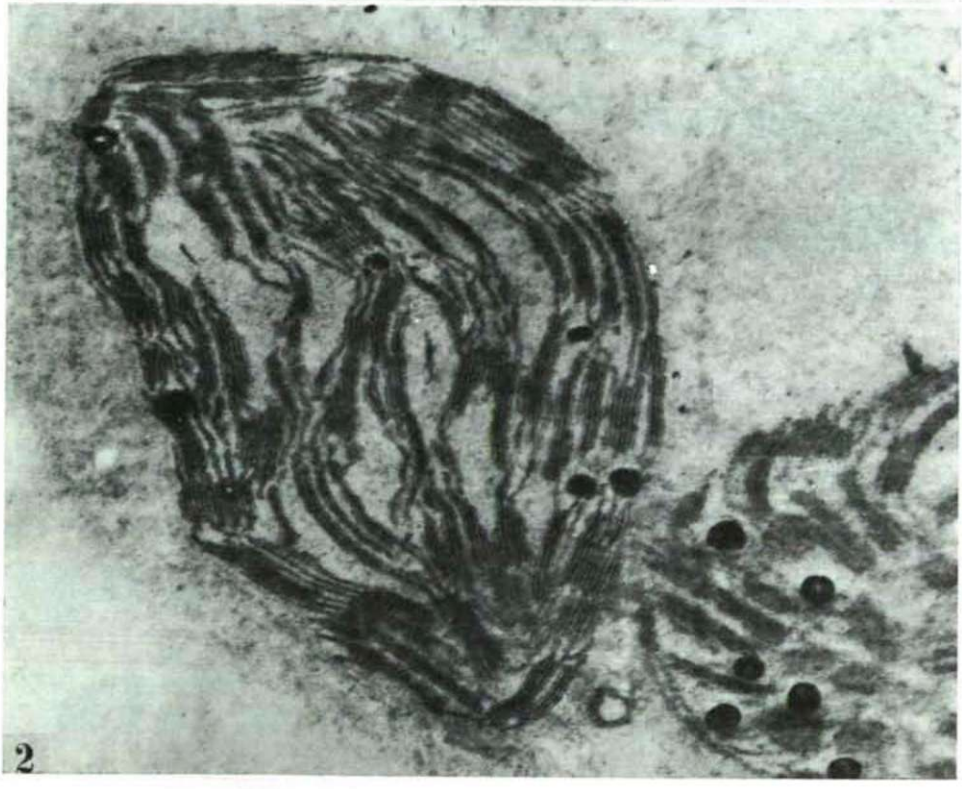

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lowest amount was found in the M chloroplasts of corn 523; in only 12 from 40 chloroplasts.

In the M chloroplasts of the leaves grown in the short 15—7.5 min LDC only a lower amount and degree of starch occurred. It was striking that only small starch granules were found in the large „starch holes". No, or only small amount of starch was observed in the granal chloroplasts of loose, swollen structure.

Discussion

The amount of internal lamella system of the chloroplasts as well as the granum formation largely depend on the light intensity.

From the examination of the light and shadow plants (ANDERSON, 1973; PRIOUL, 1973; VLASZOVA and OSZIPOVA, 1973) it can be seen that in the shadow plants the granum formation is increased, the surface of the membranes falling on a unit area is large. The dependency of granum formation on light intensity is also supported by the experiments of BOARDMAN et al. (1974) carried out on *Atriplex*. The decrease in light intensity also presumably plays a significant role in the fact that the complete length of the partitions per granum is higher in the spongy parenchymal chloroplasts, and the chlorophyll a/b ratio is lower than in the palisade parenchyma (MARÓTI and GÁBOR, 1976; MARÓTI, 1976).

The question aries that besides the same light intensity and same daily amount of light, to what extent does the length and ratio of the light-dark periods affect granum formation?

In the varying light-dark cycles where the L/D ratio is 1/50 or less (STRASSER and SIRONVAL, 1972; STRASSER and BUTLER, 1976) there is no normal granum formation. In the single and long-streched adhered double, so-called primary thylakoids developed in such a way the synthesis of the pigments, lipids and proteins, and their integration into functional units, resp. is restrained (AKOYUNOGLON, 1977; AKOYUNOGLON and ARGYRONDI-AKOYUNOGLON, 1978).

HORVÁTH and MIHALIK (1978) cultivated mustard in continuous, 3—21 h long LDC, and in such a rhythmic illumination where the 30 min of light was followed by increasing: 30, 60, 120, 240, 360, 480 min. long dark periods. In the 15 days old plants, due to the rhythmic illumination the number of grana and stroma thylakoids increased compared to the 3 h long continuous light. This result shows that in the case of a low daily amount of light and 1/7 light-dark ratio the periodical light stimulates the formation of grana and stroma thylakoids better than the continuous illumination.

On the contrary, one of the characteristic effects of our 15—7.5 min LDC experiments was that in the mesophyll chloroplasts of corns 165 and 3901 the number of grana thylakoids decreased and the intact grana consisting of many (14—35) thylakoids were missing (Fig. 1). One of the characteristic unfavourable effects of the 15—7.5 min LDC was that compared to the 16—8 h LDC — it decreased the dry matter content in the studied corns (MARÓTI and MIHALIK, 1982). The question is whether this is in connection with the daily amount of illumination, light intensity, or the length of the light-dark period?

In the 16—8 h and 15—7.5 min LDC-s the daily illumination was 16 hours, the light-dark ratio was 2/1, nevertheless, in the short cycle the leaves of the plants were

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Plate II. *Zea mays* L. *Pioneer* 523
1. light-dark cycle 16—8 h (30 000 X)
2. light-dark cycle 15—7.5 min (30 000 X)

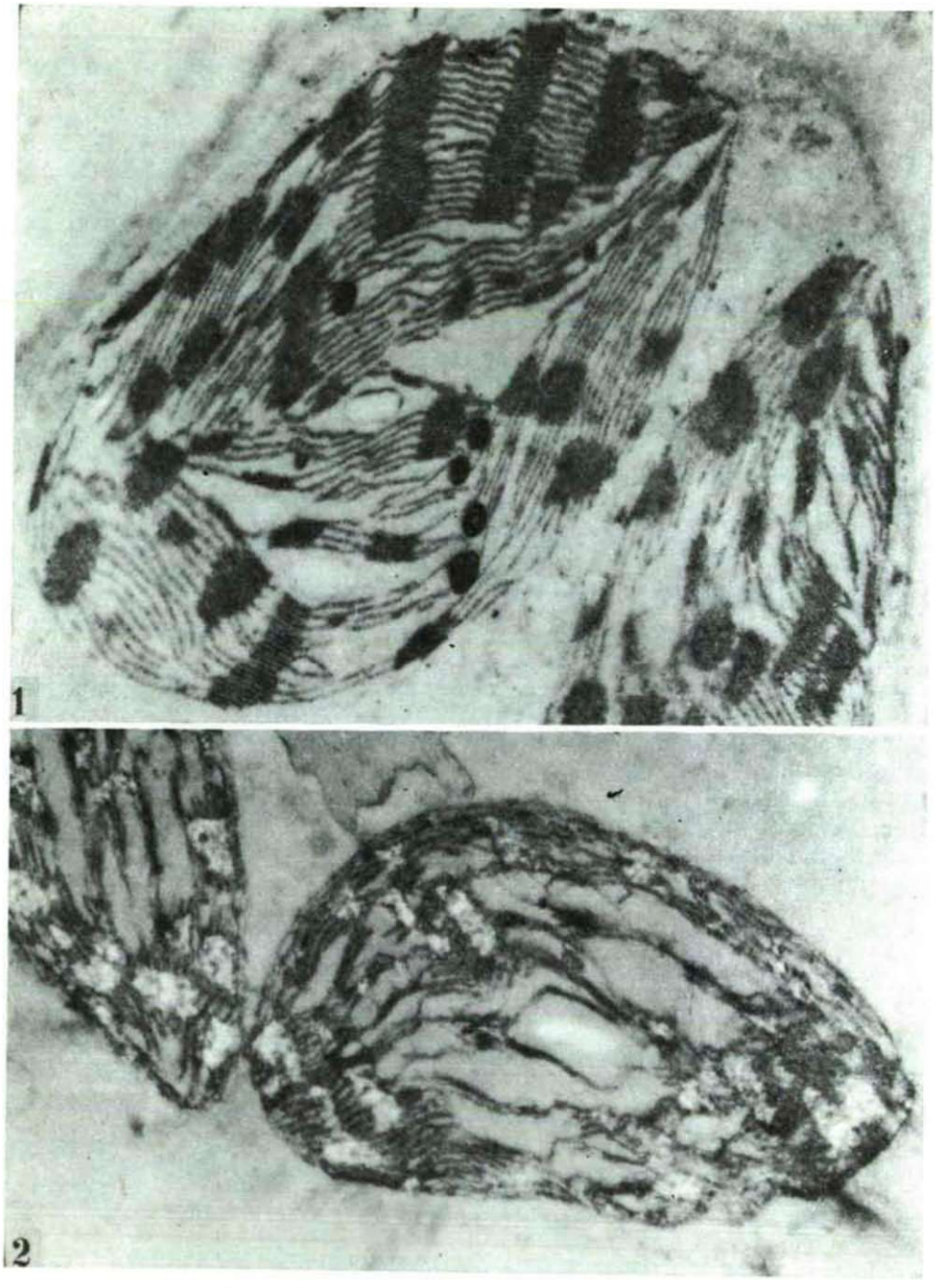

Plate III. *Zea mays* L. *Pioneer* 3901
1. light-dark cycle 16—8 h (30 000 X)
2. light-dark cycle 15—7.5 min (30 000 X)

pale green, yellowish-green. The assumption arises that the amount of light is moderate in the 15—7.5 min LDC, the leaves become etiolated, therefore normal granules do not develop.

As a matter of fact, the light intensity of 32 Wm^{-2} and the 512 Wm^{-2} amount of light is not sufficient for the normal development of the corns, however, this cannot be the cause of the unfavourable effect of the 15—7.5 min LDC, since they grew under the same amount of light as the control plants.

It has been demonstrated (MARÓTI, 1981) that the decrease in pigment-content is not due to the inhibition of synthesis, but to the destructive effect of the short cycles. In the short LCD-s firstly the pigments of the Chl-a/b protein complex become damaged:

— the amount of chlorophyll decreases, the decomposition of Chl-b is particularly significant, thus the ratio of Chl a/b increases,
— from the carotenoids, the decrease of neoxanthin and lutein indicates the destructive effect of the short cycles the best.

It can be concluded from the non-complete destruction of neoxanthin and Chl-b that only one component (that of 25—30 Daltons?) of the light-harvesting Chl a/b-protein complex (LHC) becomes deficient. It is also probable that only the amount of pigments become fewer.

On the basis of the results of THORNBER (1975) ahd SIEFERMANN-HARMS (1980), as well as our comparative pigment and electronmicroscopic studies, resp., it is assumed that the stacked membrane surface is proportional to the amount of neoxanthin. The fact is striking that relatively more becomes decomposed of the content of Chl-b and neoxanthin, lower in corn 523, the species accommodating better to the short cycle.

It is also assumed that due to the frequent dark periods in the 15—7.5 min LDC-s the tight adhesion of the grana thylakoids (MURAKAMI and PACKER, 1970; BARBER, 1976; BARBER and CROW, 1979) becomes loose and deficient, resp., because of the repeated proton efflux. Due to the deficient and looser adhesion, the neoxanthin, lutein and Chl-b found on the surface of the LDC-s become free and start to decompose. Therefore, the H^+/Mg^{2+} exchange between the interthylacoid stacking surface and the loculus may be one of the important regulators of the grana aggregation.

The observed significant increase in the amount of grana consisting of 2—4 thylakoids during the course of the 15—7.5 min LDC is brought into connection with foregoing.

The mosaic-like loosening, "decomposing" of the partition of grana containing many thylakoids can be explained by the frequently recurring dark-induced proton efflux, and the complete destruction of certain light-harvesting Chl a/b-protein complexes.

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Address of the authors:
DR. I. MARÓTI
DR. EDIT TAKÁCS
Department of Botany, A. J.
Department of Botany, A. J. University
H-6701 Szeged, P.O. Box 657, Hungary

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ANATOMICAL COMPARISON OF THE FLAG AND SECOND LEAVES OF TWO TRITICUM AESTIVUM CV. SPECIES

SZERÉN PATAKY, J. BÁLINT and I. MARÓTI

Department of Botany, Attila József University, Szeged, and Cereal Research Institute, Szeged
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Abstract

The anatomical structure of flag and second leaves of two autumn wheats: *T. aestivum* cv. GK Szeged and cv. Jubilejnaja 50 was studied by light- and with scanning- electron-microscopy (SEM). One part of the plants were grown in the glass house, the other part grew in arable land.

The flag leaves of both wheat cv. were slightly thicker, wider, their area larger, and contained cc. 20 larger and smaller intermediate bundles more than the second leaves. Compared to the second leaves, the width and thickness of the GK Szeged flag leaves, and the length and area of the Jbj 50 flag leaves were significantly larger.

On the abaxial side of the GK Szeged and Jbj 50 cv. flag leaves, above the small intermediate bundles and between the veins, the height of the mesophyll cells was smaller than in the second leaves. In the flag leaves the height of the mesophyll cells beneath the bulliform cells decreased in the case of Jbj 50, and showed almost no changes in GK Szeged, compared to the second leaves.

As a uniform characteristic, in the two wheat types the multilobed cells were more frequent in the flag leaves than in the second ones. In both leaves of Jbj 50 the multilobed mesophyll cells occurred in higner percentage than in the leaves of GK Szeged.

The flag leaves of both wheats had significantly higher number of stoma and trichoma and stronger epicuticular wax covering on the adaxial than on the abaxial surface.

On the basis of the alterations in the lobed structure of the mesophyll cells and our previous studies it is assumed that in the chloroplasts of flag leaves the ratio of cyclic per linear electron transport is higher than in the chloroplasts of the second leaves.

Key words: *Triticum aestivum*, flag and second leaves, morphology, lobed mesophyll cell, epidermis.

Introduction

In the recent years sereval authors (CHONAN, 1965, 1966, 1970; KHAN and TSUNODA, 1970, 1971; AUSTIN et al., 1982; PARKER and FORD, 1982) demonstrated reaationship between the anatomical structure and photosynthetic activity of wheat leaves. The majority of the authors (KHAN and TSUNODA, 1970; EVANS and DUNSTONE, 1970; AUSTIN et al., 1982; PARKER and FORD, 1982) connect the intensity of photosynthesis with the larger surface of the leaves in the case of wheat, too, which — according to these authors — facilitates the diffusion of carbon dioxide into the mesophyll cells.

The question is how the larger internal surface and more intensive carbon dioxide uptake of the diploid *T. urartu* leaves could be fit together with the larger productivity of the hexaploids.

AUSTIN et al. (1982) isolated chloroplasts and protoplasts from diploid, tetraploid and hexaploid wheat leaves and demonstrated that the ratio of the light-dependent oxygen evolution was close to similar. Authors assumed that the differences in photosynthesis between the intact leaves may originate from the anatomical variations of the leaves. On the basis of the studies on the genotype of 15 diploid, tetraploid and hexaploid wheats, authors did find positive relationship between the vein density and maximum photosynthetic rates.

This observation also calls attention to the intensity in transport of the assimilates. The question arises, however, whether the higher amount of assimilates are transported because there are more veins, or because a larger number of assimilates develop, which are capable of transport and vein-formation, resp.

Opinions vary regarding the place of formation of the organic matter accumulated in the yield and the rate of participation of certain organs. On the basis of his experiments carried out with comparative defoiling, BONSTRA (1937) drew the consequence that from the assimilates found in the grains 75% is produced in the upper part of the wheat: in the spike (34%), the upper internode (12%), the flag leaves (13%) and sheath (16%).

According to the studies of SIMPSON (1968) and FOCKE (1973) in the majority of wheat species the spike shows the tightest connection with the yield, in point of view of assimilate-formation, and this is followed by the flag leaf and the rest of the leaves.

The role of the spike is coming more and more into the foreground with the decrease in the height of the species, while that of the flag leaf and the other leaves is changing according to variety (BEKE, 1977).

According to the studies of NALBORCZYK (1978), in the case of wheat the spike contributes to the development of the total photosynthetic products (dry matter) in 9.3%, the upper internode in 16.2%, the flag leaf lamina in 36.6%, the flag leaf sheath in 7.0%, the lamina beneath the flag leaf in 18.8%, the second leaf sheath in 7.1%, the third leaf and the internode underneath in 4.2%.

On the basis of the studies by LEDENT and POCHET (1978) the length of the veins counted per unit area of the flag leaves, and the number of tracheas, resp. show tighter correlation with the yield than the area, width or weight of the flag leaves.

The questions raised cannot be solved by histo-comparative studies on the flag leaves and leaves under these, but — besides the fact that there are no such studies — indicate the histological examination of the two upper leaves of two hexaploid wheat types having various productivity (Jubilejnaja 50, GK Szeged).

The literary data are in agreement in that the spike has significant role in the formation of assimilates stored in the grains; this is followed by the flag leaf and with great difference, by the second leaf beneath this.

In the present paper we study on one part how this variation is manifested in the tissue structure of the leaves, and on the other part, a new relationship is presumed between the structure of the mesophyll cells and photosynthesis.

Materials and methods

PLANTS AND EXPERIMENTAL CONDITIONS

The experiments were carried out with the autumn types of Triticum aestivum L. commonly cultivated in Hungary; cv. GK Szeged and cv. Jubilejnaja 50. The GK Szeged is a wheat type which is intensive and belongs to the group of early ripening, its largescle productivity is 7.0—8.5 t ha^{-1}. The Jubilejnaja 50 is a wheat type which can be securely cultivated, belonging to the group of moderately early ripening. Its large-scale productivity is 6.0—6.5 t ha^{-1}.

For one of the experiments — to determine the thickness of the flag leaves and second leaves beneath these; the measurements of the mesophyll cells; as well as the lobe number and stoma number and measurements — the two types of wheat were grown in Henssler-type climate-house according to the followings: the two-leaved plants were placed into pots with diameters of 14 cm (4 plants/pot) after 50 days of vernalization and were grown in homogenized soil supplied well with nutriment. Following unpotting the plants grew for 10 days at 13—15 °C under 50—55% relative humidity.

Then the temperature was raised to 21—23 °C and the humidity varied between 60—65%.

The daily 14 h illumination of the plants was ensured by 400 W Phillips HLRG lamps (30 W/m^2) in January, February and March.

For the other experiment — to determine the measurements of the flag and second leaves; the number and density of the bundle types; and the amount and measurements of the stoma — the

wheat plants were grown in the fields, in the Lower ground of the Cereal Research Institute at Szeged. The time of sowing was October 14, 1980, performed with 400 seeds/m² close setting in plots of 10 m².

In case of both experiments the flag and second leaves were collected for anatomical examinations at the time of flowering.

Determination of the measurements of leaf laminae and amount of veins

50—60 plants grown in the fields were taken in order to determine the length, width and area of the flag and second leaves. After the determination of the fresh weight drawings were made from the laminae of the leaves and the area was recorded by weighing.

To determine the type and amount of the leaf veins 1 cm long pieces were cut from the centre part of the leaves (about 12 cm from the base) and these were refined in 5% sodium hypochlorite solution for 24 h, then washed in thin acetic acid. The refined leaves were placed into photographic enlarging apparatus and projected (Fig. 2.)

For labelling the various bundle types the works of Patrick (1972) and Kuo et al. (1974) were applied: the midrib was the largest, followed by the 6 large laterals (Ll), and then the four smaller laterals (Sl). The amount of larger lateral bundles was generally 10 (5 oh one side), among these varrying numbers and sizes of large intermediate (Li) and small intermediate (Si) bundles were found. The leaf laminae thickness was measured in the case of the Ll, Li, Si bundles, and in the direction of the bulliform cells situated in the neighbourhood of these bundles (Llb, Lib, Sib) (Fig. 1).

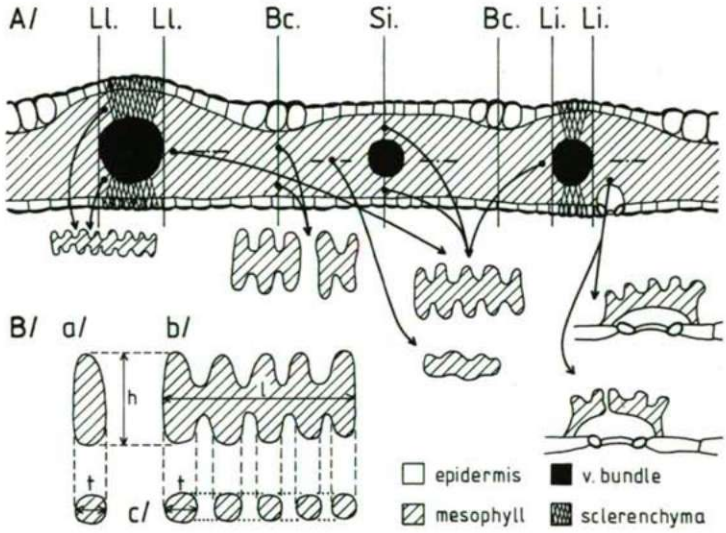


Fig. 1. A) Diagram of the transverse view of the leaf, places of measurings of the height, width (lobe diameter) of mesophyll cells, and longitudinal view of mesophyll cell forms characteristic to the places of measurings (Ll: large lateral; Li: large intermediate; Si: small intermediate bundles; Bc: bulliform cells). B) Transverse (a) longitudinal (b) and top view (c) diagram of the mesophyll cell (h = height of cell; t = cell thickness and lobe diameter; l = cell length).

Studies on the mesophyll cells

For the mesophyll studies samples were taken from the centre part of the flag and second leaves of four plants (about 12 cm from the base). The transverse and longitudinal sections of 25—30 μm were prepared by Leitz Landa type freezing microtome. Zeiss NU_2 light microscope was used for the mesophyll cell measurings.

In the transverse sections of the leaves measurements were taken of the height (h) and thickness (t) (width) of the mesophyll cells. The width of the cells correlated with the diameter of the lobes (Parker and Ford, 1982).

The mesophyll cells were measured at the following points:

1. the large lateral (Ll);
2. large intermediate (Li);
3. small intermediate (Si) bundles and
4. in the direction of the bulliform cells (Bc) situated between them, on the reverse and surface sides, resp. (Fig. 1).

30—30 cells were measured at each point, therefore, a total of 240 cells were measured from the surface and reverse sides.

In the leaf-longitudinal sections the lobes of the mesophyll cells were counted. The length of the cells was not measured, however, this could be calculated from the diameter and number of the lobes. During the course of one of the measurings (without selection) about 200 mesophyll cell lobes were counted. In the case of the other measuring (about 40—50 cells) the lobe number of the mesophyll cells beneath bulliform cells was distinguished.

LIGHT-AND SCANNING- ELECTRON-MICROSCOPIC STUDIES ON THE EPIDERMIS

The 1 cm^2 pieces gained from the centre part of the flag leaves were fixed with so-called Karnovsky solution (KARNOVSKY, 1965) containing paraformaldehyde-glutaraldehyde for a period of 6 h at +4 °C. Then after washing in phosphate buffer (0.1 M pH 7.2) for 18 h the samples were dehydrated linearly in acetone and dried at carbon dioxide atmosphere by critical point method (Polaron).

The dried samples were then studied after gilding in a TESLA BS 300 type scanning-electron-microscope.

In order to measure the amount of stoma and the length of the guard cells excoriations were prepared from the centre part of the leaves. The average of the data of 50 visual fields (M:10×16) per sample was evaluated in the epidermis studies.

Results and discussion

MORPHOLOGICAL CHARACTERIZATION OF THE FLAG AND SECOND LEAVES OF GK SZEGED AND JUBILEJNAJA 50

The flag leaves of both wheats were slightly wider, their area larger than that of the second leaves. The area of the upper two leaves of GK Szeged was larger than those of the Jubilejnaja 50. In the case of both wheat types the length of the flag leaves varied oppositely compared to the second leaves. The flag leaves of GK Szeged were shorter, those of Jubilejnaja 50 were longer than the second leaves (Table 1).

Table 1. Measurements and number of bundles of the flag and second leaves

	GK Szeged flag leaf	2 nd leaf	Jubilejnaja 50 flag leaf	2 nd leaf
Measurements of the leaf:				
Lenght (cm)	25.1±3.2	27.2±2.8	27.6±3.4	25.8±2.6
Width(cm)	2.1±0.3	1.9±0.8	1.8±0.2	1.7±0.2
Area (cm^2)	44.7±5.2	43.6±4.6	41.7±5.1	35.8±3.5
Type of bundles:				
No. of Ll+Sl (No/leaf)	11	10	10	9
No. of Li+Si (No/leaf)	52	32	47	28
Length of Ll+Sl (cm/cm^2)	5.23	5.26	5.55	5.29
Length of Li+ Si (cm/cm^2)	24.76	16.84	26.11	16.47

Types of bundles: Ll large lateral; Sl small lateral; Li large intermediate; Si small intermediate. The measurings are the average data of 50 completely developed (flowering) plants grown in the field. The leaf width means the greatest width.

Great difference could be observed in the number of large and small intermediate bundles and the length per unit surface of the flag and second leaves (Table 1).

In the flag leaves, close to 20 more intermediate bundles were found in case of both wheats than in the second leaves (Fig. 2).

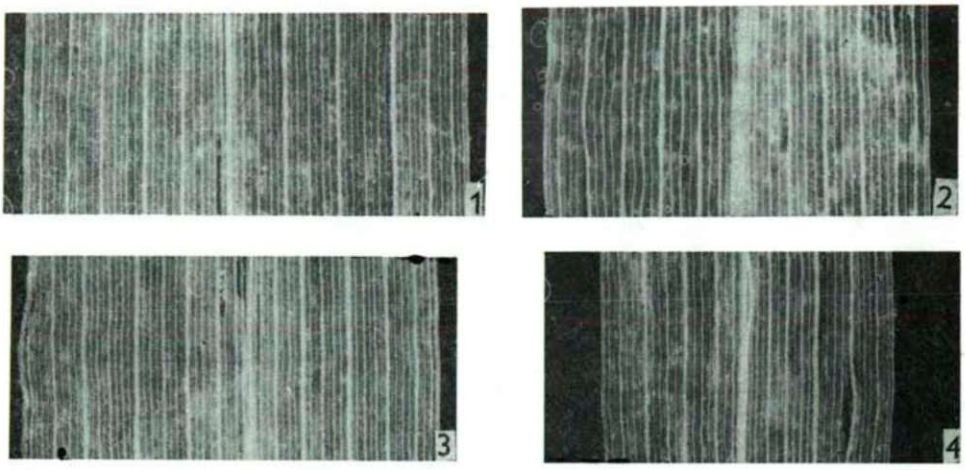


Fig. 2. Arrangement of vein types in the flag (1, 3) and second leaves (2, 4) of GK Szeged (1, 2) and Jubilejnaja 50 (3, 4) in the whole width of the leaves (same magnification).

The area and width of the flag leaves showed strongly negative, while the number of veins per cm showed positive correlation with the net photosynthesis rate (AUSTIN et al., 1982). The value of the bundle length per cm^2 was in agreement with the number of veins/width of leaves in cm (Table 1).

The width of the GK Szeged flag leaves; and the area of the Jubilejnaja 50 cv. flag leaves significantly increased in comparison to the second leaves. Nevertheless, this change was only of small degree compared to the increase in the amount of the veins.

Although the amount of CO_2 uptake under unit of time by the unit of leaf surface showed negative correlation with the width of the flag leaves (AUSTIN et al., 1982); it is probable from the viewpoint of transport of the assimilates that the flag leaves of GK Szeged cv. — which are winder and contain more veins — are more favourable than the narrower leaves of the Jubilejnaja 50 cv.

COMPARISONS BETWEEN THE THICKNESS OF THE FLAG AND SECOND LEAVES

The thickness of the upper two leaves was not identical with the complete width of the lamina. Different values were measured in the case of the variously large veins, and between them, resp., and the thickness showed a decrease towards the edge of the leaves.

It can be seen from the data of Table 2 that in case of both wheats the flag leaves were thicker than the second ones, towards the large lateral (Ll), large intermediate (Li) bundles and the bulliform cells between these. No difference was observed in the thickness of the flag and second leaves of Jubilejnaja 50 cv. in the small intermediate (Si) bundles and the bulliform cells found between them. Significant differences between the two leaves could only be observed at every measuring point in the GK Szeged cv. (Fig. 3, Table 2).

Table 2. Thickness of flag and second leaves in the direction of the Ll, Li, Si bundles and the bulliform cells between them (Llb, Lib, Sib) (See abbreviations in Table 1).

Place of measurings	Leaf thickness (μm) GK Szeged flag leaf		GK Szeged 2 nd leaf	Jubilejnaja 50 flag leaf		Jubilejnaja 50 2 nd leaf
Ll	260.2±5.8	***	238.8±7.5	245.6±0.7	***	220.1±2.4
Llb	209.3±5.2	***	194.7±3.9	189.9±0.8	**	178.8±3.9
Li	218.2±3.6	***	207.0±0.3	197.1±1.6	***	183.2±7.5
Lib	185.6±2.8	***	175.7±0.8	164.5±2.3	NS	163.7±4.7
Si	180.4±5.5	***	173.6±1.2	161.3±5.5	NS	162.9±3.9
Sib	162.9±3.9	***	146.2±4.7	131.9±4.7	NS	133.1±5.9

** : SZD significantion on 1 % level
***: SZD significant on 0.1 % level
NS: no signification

The flag leaves of GK Szeged cv. were expressedly thicker than those of Jubilejnaja 50. It was striking that this was not manifested in the dry weight per unit surface of the flag leaves, since the dry weight of these leaves in GK Szeged was 525 mg dm^{-2}, and the dry weight of Jubilejnaja 50 was 531 mg dm^{-2}. On the contrary, the fresh weight of GK Szeged flag leaves was $2133^{\pm 30}$ mg dm^{-2}, and $2052^{\pm 38}$ mg dm^{-2} in case of Jubilejnaja 50.

The thickness of the flag leaves showed tight correlation with the diameters and areas of the bundles, and the measurements of the mesophyll cells, resp. (LEDENT and POCHET 1978). In the case of the studied two wheats the greater thickness of the flag leaves could firstly originate from the larger size of the veins.

THE MESOPHYLL OF THE FLAG AND SECOND LEAVES

SHAPE AND ARRANGEMENT OF THE MESOPHYLL CELLS

On the transverse section of the wheat leaves and from the perpendicular surface view of the leaves, the mesophil cells under the surface and reverse epidermis were greatly similar to the typical palidase cells (Plate l, Pictures 1, 2, 3,). However, the longitudinal section of the leaves showed that thet mesophyll cells were elongated in a parallel manner with the veins of the leaves and were lobed (TUAN, 1962; CHONAN, 1965; PARKER and FORD, 1982). Therefore, the height and thickness of the mesophyll cells (also corresponding to the lobe diameters) could be measured on the transverse section of the leaves, and the length of the cells, as well as the number of lobes could be recorded from the longitudinal sections (Fig. 1).

The shape, measurements and number of lobes of the arm palisade-like lobed mesophyll cells differed, compared to the cells beside and between the veins. The sclerenchyma edges above and below the larger bundles were bordered by the longest and shortest mesophyll cells having the largest number of lobes. The external thin-walled bundle sheaths were joined by long, many-lobed mesophyll cells, which resembled palisade cells arranged radiately on the transverse sections of the leaves (Plate 1,

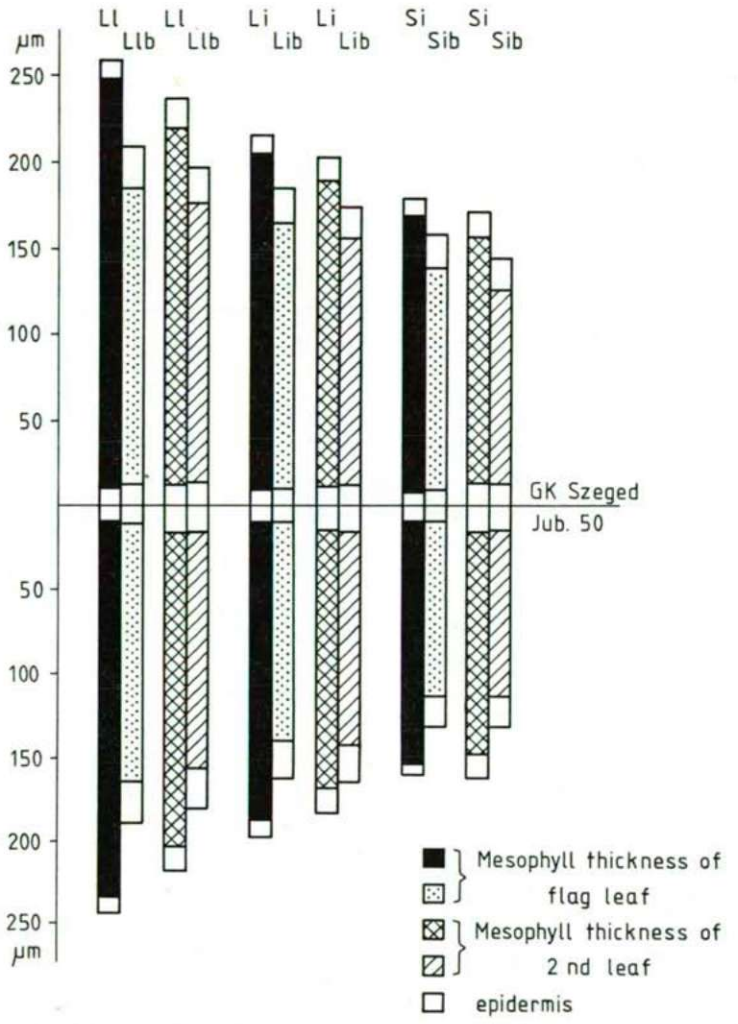
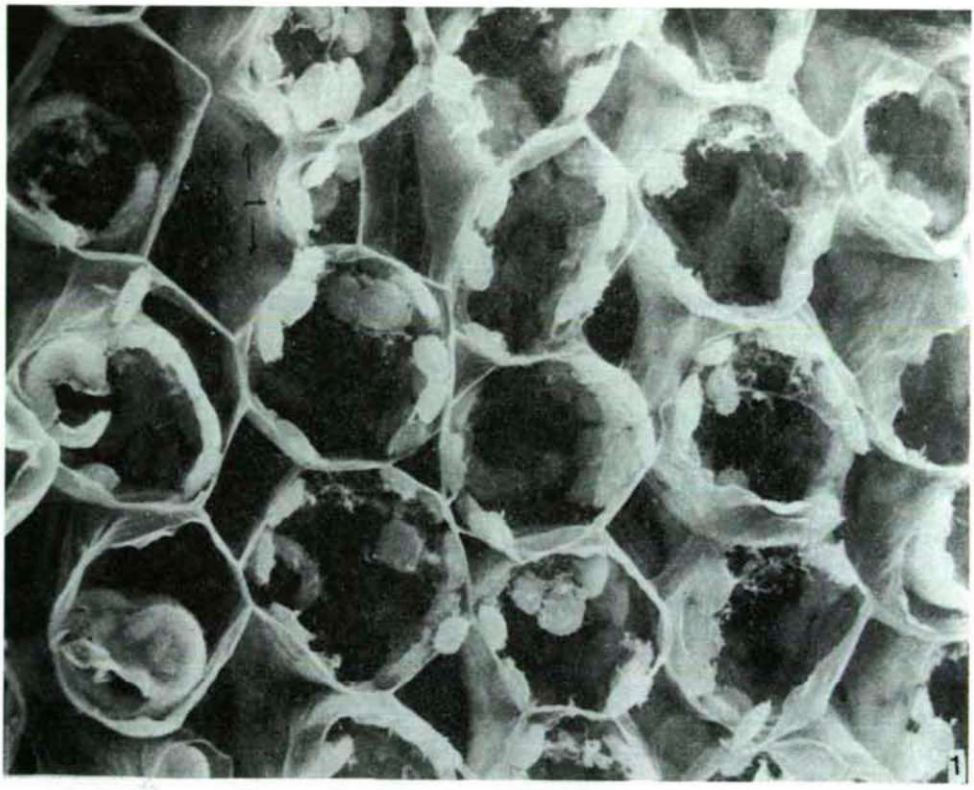


Fig. 3. Changes in thickness of flag and second leawes along the various types of bundles and the bulliform cells between them. (See abbreviations in Table 1).

Pictures 2, 3). The radiately arranged mesophyll cells were in tight junction with each other, the fewest among them were the air-filled intercellular spaces. As it is known, the largest amount of intercellular spaces in the unit volume of the mesophyll can be found under the stoma layers (Plate 1, Picture 2), where characteristically assymetric mesophyll cells with 2—5 lobes can be detected (Fig. 1).

Under the bulliform cells the shorter mesophyll cells having longer lobes, however, showed perpendicular arrangement on the surface of the leaves. The rims of the leaves were lined with sclerenchyma having several cell layers (as are usually the leaves of grass) METCALFE, 1960).




Li
b
r
Si


r
Si
b

MEASUREMENTS OF THE MESOPHYLL CELLS

On the transverse section of the leaves, under the reverse and surface epidermis, resp., the height and width of mesophyll cells were measured beneath the various sized bundles and bulliform cells found among them (Table 3, Fig. 1). It could be seen from the results of the measurings that the mesophyll cells between the veins the flag second leaves were essentially higher and wider (thicker) than those beside the veins.

Compared to the second leaves the height of the mesophyll cells was lower, and the width (lobe diameters) wider under the bulliform cells in the flag leaves of the Jubilejnaja 50 cv. On the contrary, in the flag leaves of GK Szeged cv. the mesophyll cells between the veins showed only slight changes in height and the width was found to be decreased in comparison to the second leaves.

In the height of the mesophyll cells (Table 3) it could not be manifested unambiguously that the flag leaves were thicker than the second leaves (Fig. 3). The greater

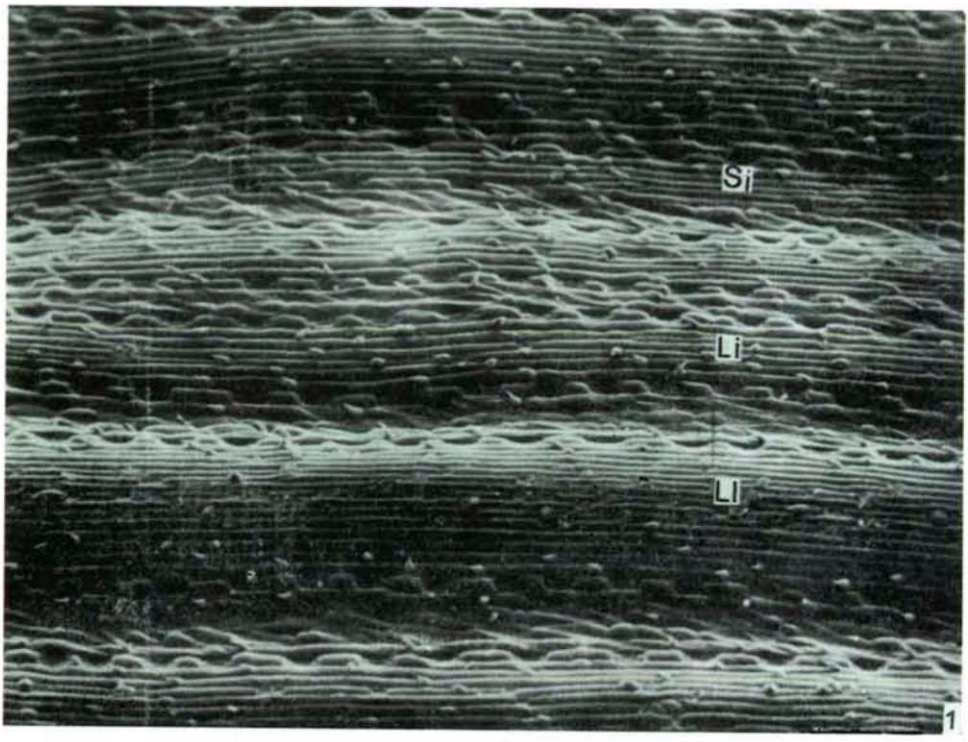
Table 3 Measurements of mesophyll cells in flag and second leaves

		Flag leaf		2 nd leaf	
		Adaxial position	Abaxial position	Adaxial position	Abaxial position
GK Szeged					
Ll		25.5±4.5*	26.6±4.6	28.3±3.6	28.1±3.8
Li	cell height	26.6±3.4	24.3±3.7	26.4±3.6	26.4±3.8
Si	cell height	29.4±2.6	24.6±3.4*	27.2±4.8	27.0±7.0
Bc		45.0±3.0	39.2±4.8	44.2±5.8	41.9±5.9
Ll		10.6±3.4	10.6±1.4*	10.4±1.5	11.9±2.0
Li	cell thickness	9.6±2.4	9.6±2.4	10.2±3.7	10.5±4.8
Si	cell thickness	9.8±2.2	9.0±3.0*	9.2±2.7	10.5±1.5
Bc		11.4±2.6*	12.2±1.8	14.4±3.6	13.3±2.7
Jubilejnaja 50					
Ll		27.7±4.3*	27.4±4.6	24.3±1.7	25.6±4.4
Li	cell height	25.0±3.0	26.9±3.1	26.2±3.8	27.8±2.2
Si	cell height	29.1±5.1	26.5±3.5*	29.6±2.4	29.5±3.5
Bc		33.8±6.2*	31.4±7.4*	44.6±3.4	37.2±5.8
Ll		10.9±1.1	10.4±1.6	11.0±3.0	10.6±1.4
Li	cell thickness	9.8±2.2	10.7±1.3*	10.2±1.8	9.4±2.6
Si	cell thickness	10.6±1.4	10.0±2.0*	10.6±1.4	8.8±1.2
Bc		14.1±3.9*	12.6±3.4*	12.2±1.8	9.5±1.5

Measurements were made on the transverse section of the leaves from the midrib to the leaf margin: in the direction of the large lateral (Ll), large intermediate (Li), small intermediate (Si) bundles and the bulliform cells (Bc) between them, under the reverse and surface epidermis of the mesophyll cells. The height and thickness of the cell were measured. (The cell thickness or width is equivalent to the lobe diameter).

*: The value measured in the flag and second leaves is significant on 5% level.

Plate I. Form of mesophyll cells: Picture 1: SEM picture of the lobes of mesophyll cells filled with chloroplasts (1), with the surrounded intercellulars, from a view perpendicular to the abaxial surface. (GK Szeged flag leaf, M: ~1250 x). Pictures 2.3: Light microscopic picture of transverse section of Jubilejnaja 50 flag (Picture 3, M: 640 x) and second leaf (Picture 2, M: 200 x) with small lateral (Sl), large intermediate (Li) and small intermediate (Si) bundles, mesophyll cells under the radial (r) and bulliform (b) cells.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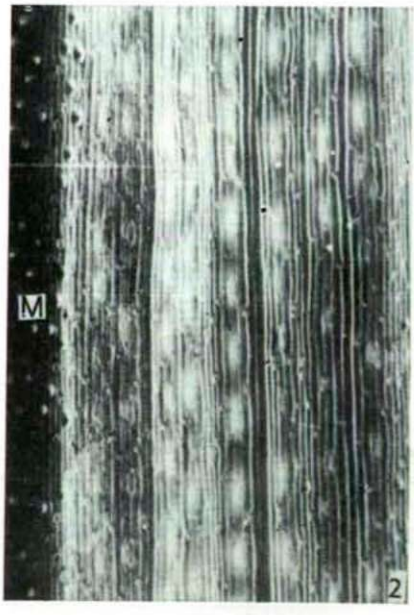
Li
Li
1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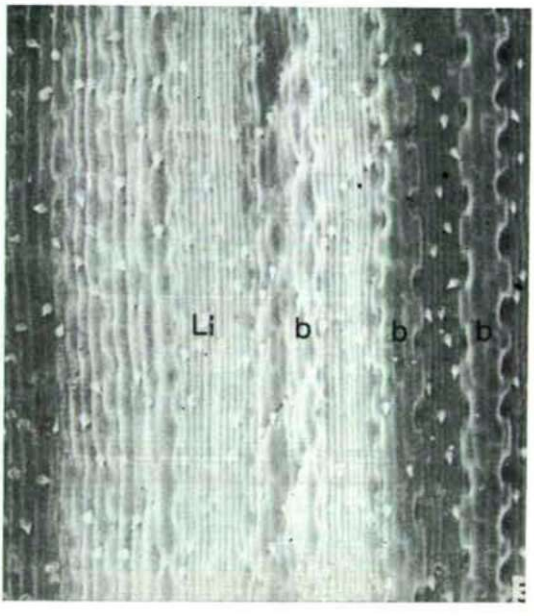
Li
b
b
b

thickness of the GK Szeged and Jubilejnaja 50 cv flag leaves firstly originated from the larger size of the large lateral and large intermediate bundles.

In the case both studied corn types the height of the mesophyll cells on the abaxial side of the flag leaves, under the small intermediate bundles and between the veins (Table 3) showed a decrease compared to the second leaves. The determination of LEDENT and POCHET (1978) that the thickness of the flag leaves showed positive correlation with the measurements of the mesophyll cells is not considered by us to be exact, and our measurings did not support this. The height of the mesophyll cells showed a decrease, and the length an increase on the effect of weak light intensity (FRIEND and POMEROY, 1970).

The light conditions of the flag leaves were not worse than those of the second leaves, nevertheless the centre parts of the leaves were rather of a horizontal position and therefore the abaxial side may be more shadowed than that of the second leaves, which slope better. On the abaxial side of the Jubilejnaja 50 cv flag leaves the height of the mesophyll cells under the bulliform cells also showed a more significant decrease in comparison to the second leaves (Table 3), and this result cannot be explained by the changes in light intensity.

THE NUMBER OF LOBES PER CELL

The percental distribution of the frequency of the number of lobes per cell was studied in two ways on the longitudinal sections of the leaves. On the one hand the lobes of 200 randomly-selected mesophyll cells were counted on the longitudinal sections (Fig. 4, first A column row). In the other „casual samples" only the lobes of the mesophyll cells beneath the bulliform cells (cc. 40 cells were counted (Fig. 4, second B column row).

It was a uniform characteristic in the case of both wheat types that the multilobed mesophyll cells were more frequent in the flag leaves than in the second leaves (Fig. 4). For example, in the GK Szeged cv. flag leaves the 8-lobed mesophyll cells situated under the bulliform cells occurred with a frequency of 45 %, and 13 % in the case of the second leaves. This difference in the number of lobes was more intensive in the Jubilejnaja 50 cv. flag and second leaves (Fig. 4).

The question is: what anatomical characteristics is the increase in the number of lobes per cell of the flag leaves connected with?

It is known that in the upper leaves of wheat the mesophyll cells are larger and have more lobes than in the lower leaves (CHONAN, 1965). According to our opinion the mesophyll cells of flag leaves are not in general larger, but rather longer than those of the second leaves. The mesophyll cells of the flag leaves of the hexaploid *T. Aestivum* cv. Professeur MARCHAL are twice as high and double-lobed than those of the diploid T. urartu (PARKER and FORD, 1982). On the effect of the decrease in light intensity both the length and number of lobes of the mesophyll cells increased (FRIEND and POMEROV, 1970).

According to our studies one of the main causes of the increase in lobe-number of the flag leaves (compared to the second leaves) may be the greater elongation of the mesophyll cells and the decrease in the height of the cells. In the flag leaves, the

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Plate II. SEM picture of the arrangement of the cell types forming the ad- and abaxial epidermis of the flag leaves: GK Szeged adaxial (Picture 1) and abaxial (Picture 2) area; Jubilejnaja 50 adaxial (Picture 3) area (M: ~50 x).
Ll = large lateral, Li = large intermediate; Si = small intermediate bundles, b = bulliform cells, M = midrib,

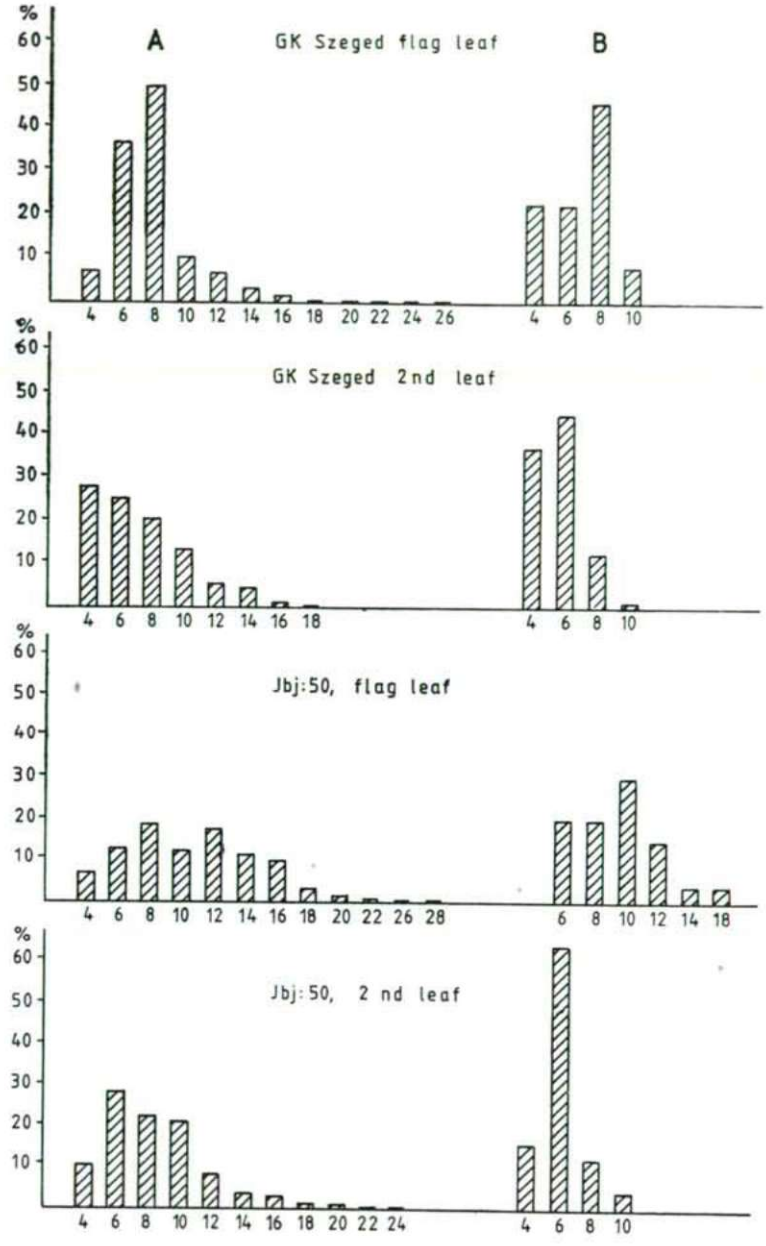


Fig. 4. Percental distribution of the lobe number of mesophyll cells in the flag and second leaves of GK Szeged and Jubilejnaja 50. Casual distribution (randomly selected sampling) from the mesophyll of the whole leaf (A) and only from the part of the leaf under the bulliform cells (B).

other cause of the higher incidence of many-lobed mesophyll cells is with all probability that the veins of these leaves (number of veins per cm of leaf width) show significantly greater density than in the second leaves. Therefore, the long, many-lobed

mesophyll cells situated radiately along the veins can be found in higher, while the shorter ones with fewer lobes situated between the veins can be observed in smaller percentage in the mesophyll in the case of flag leaves than in that of the second leaves.

Definite difference could be detected betwween the two wheat types concerning the percental distribution of the lobe numbers per cells. The many-lobed mesophyll cells were more frequent in both leaves of the Jubilejnaja 50 cv. than in the leaves of the GK Szeged cv. For example, beneath the bulliform cells the mesophyll cells having ten or more lobes were found to occur with a frequency of 58% in the Jubilejnaja 50 cv. flag leaves, and with a freqeancy of 7% in the GK Szeged cv. flag leaves.

COMPARISON BETWEEN THE ADAXIAL AND ABAXIAL SURFACE OF THE FLAG LEAVES

The two surfaces basically differed from each other in cell types, measurements, arrangement, stoma- and trichome amount, density of silica cells and epicuticular wax needles (Table 4).

Table 4. Changes of measurement stoma— and trichome number in guard cells

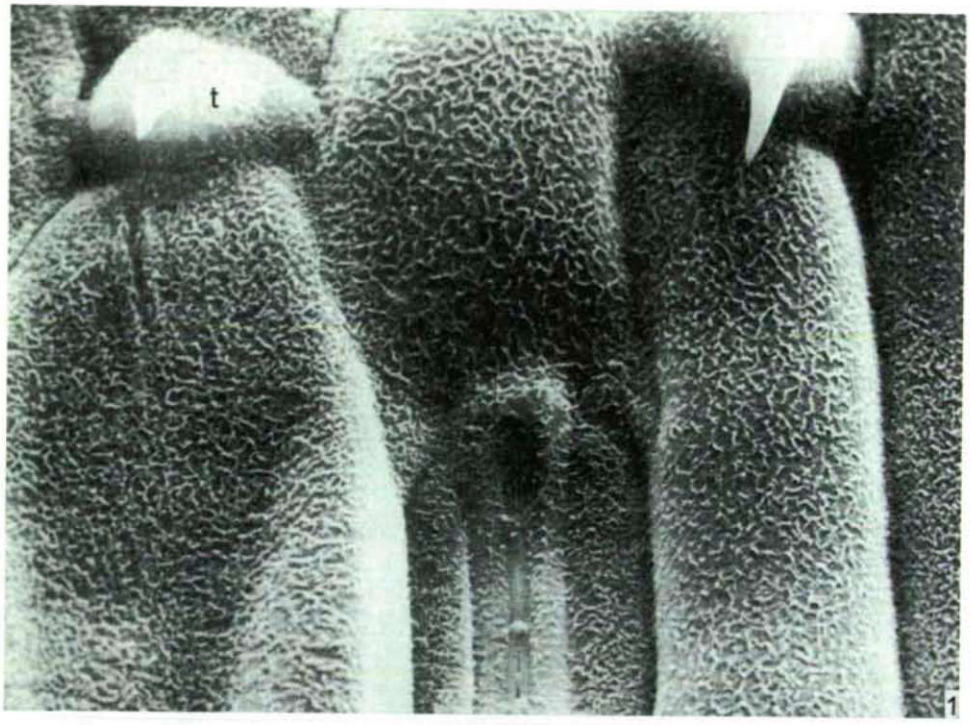
	GK Szeged, Flag leaf			Jubilejnaja 50, Flag leaf		
	Adaxial position		Abaxial position	Adaxial position		Abaxial position
In conditioned climate room						
stoma guard cell length μm	60	***	55	60		61
stoma number/cm^2	4696	***	4102	4075	***	3016
trichome number/cm^2	3440		1452	3482		1581
In the fields						
stoma guard cell length μm	60	***	55	60	***	55
stoma number/cm^2	6289	***	4809	5141	***	3756

*** = SZD significant on 0.1% level.

The nature of the adaxial surface was determined by two cell types: the epidermis cells of the costal field covering the veins, protruding above the large veins; and those of the intercostal field, resp., as well as the sections of bulliform cells (Plate 1, Picture 2; Plate 2, Picture 1; Plates III. IV, Picture 2).

Narrower epidermis cells were found to extend over the sclerenchyma cells connected with the larger veins, than over the smaller veins mesophyll cells between the veins. The stoma — between the eveins — were also arranged in longitudinal rows. The bulliform and sclerenchyma-covering epidermis cell rows were also free of stoma (Plate II, Pictures 1, 3). The larger veins were protruded on the adaxial surface, due to which the surface was slightly undulatory (Plate I, Picture 2; Plate II, Pictures 1, 3). Silica cells and needle-sharp trichomes leaning to the side were sporadically found among the epidermis cells (Fig. 5., Plate II, Picture 1., Plates III, IV., Pictures 1, 2). Longitudinal cuticule striation and compactly situated epicuticular wax needles were observable on the cell surfaces (Plate IV., Picture 2, Fig. 5).

The abaxial surface was characterized by the alternation of sections made up of epidermis cells, narrower and longer above the larger veins, and wider above the mesophyll cells (Plate II, Picture 2, Plate V. Picture 3). The various types of veins had



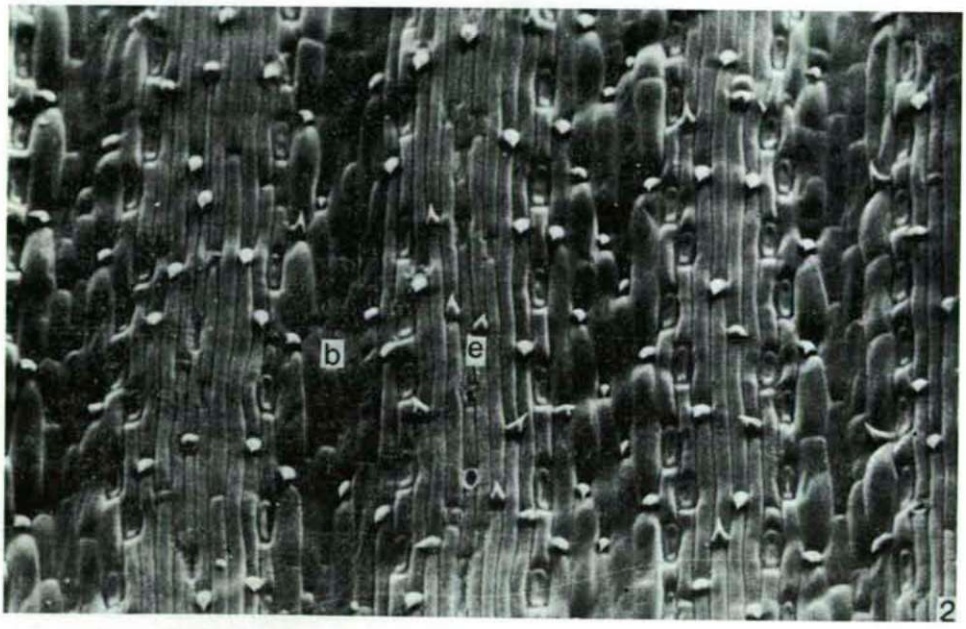


Plate III. SEM picture of sections formed by epicuticular wax needles (Picture 1, M: ~1050 x) and bulliform (b), as well as epidermis cells (e) (Picture 2, M: ~100 x). t = trichome.

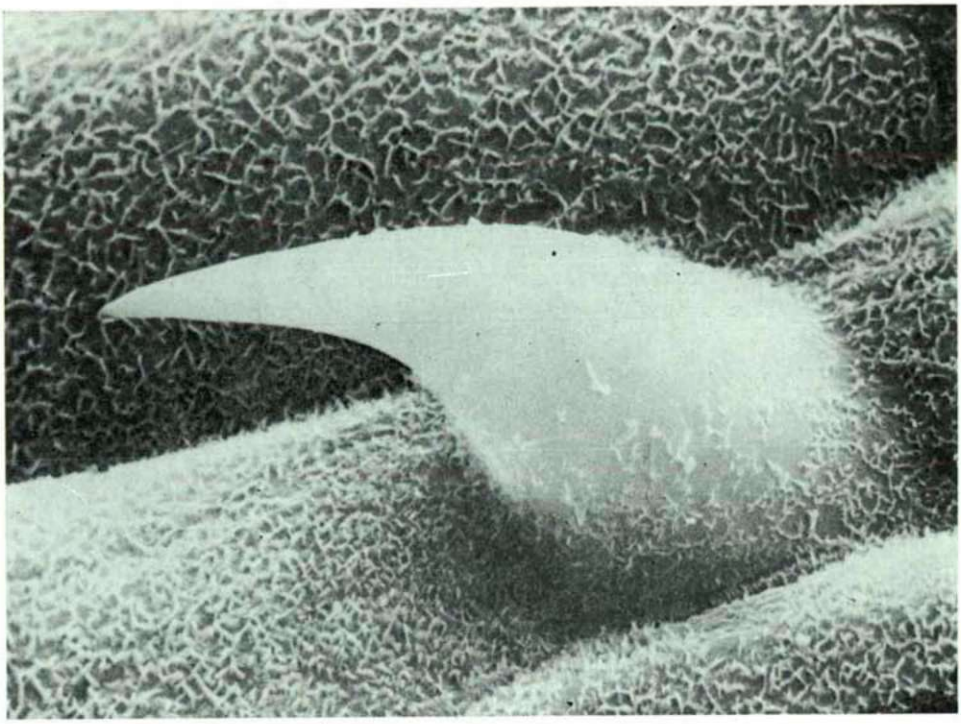


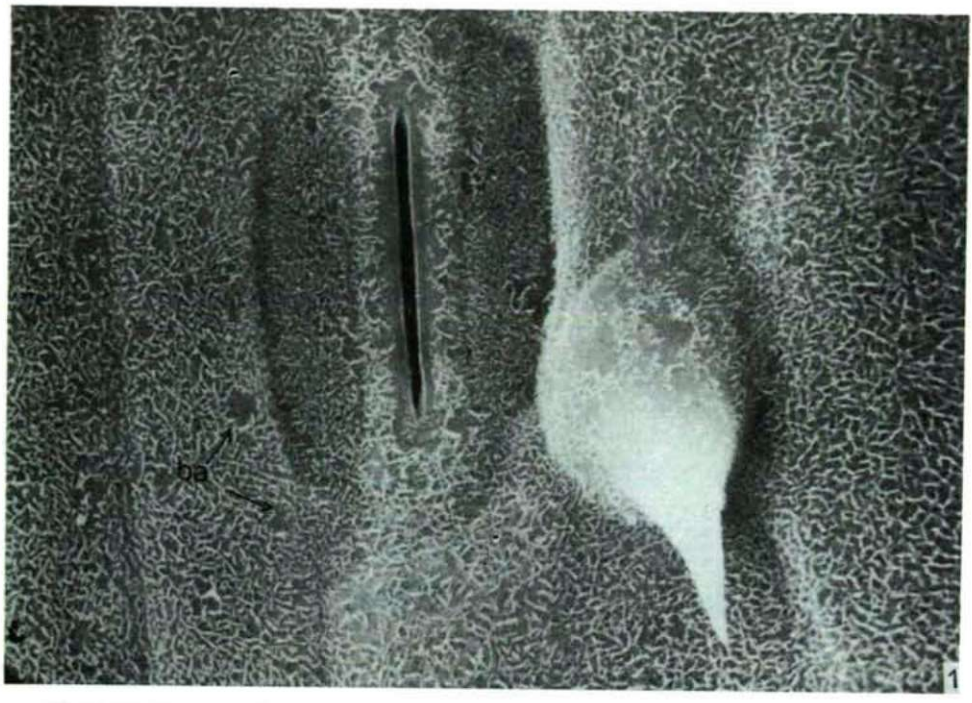
Fig. 5. Adaxial surface in GK Szeged cv. — wax needles, unicellular trichomes. M = ~ 2500 x.

determinant role, as is generally characteristic of the epidermis of grass (METCALFE, 1960; ESAU, 1969; WOLCSÁNSZKY, 1972). The main vein was strongly protruded, the larger veins only slightly; thus this surface was almost even compared to the adaxial side (Plate 1, Picture 2 Plate II, Picture 2).

The trichomes of the abaxial surface were bluntly conoid, observable above the larger veins. The main vein was the most densely covered by them. The greater density of the silica cells (Plate II, Picture 2, Plate V. Picture 2. 3) and the scarce epicuticular wax-covering (Plate V, Picture 1) were characteristic features. The epidermis above the sclerenchyma (costal field) was stoma-free, which was well observable on polarization electron-microscopic pictures (Plate V, Picture 2). On both surfaces the stomata were slightly subsided.

Great differences in stoma number were detected regarding both types between the plants grown in the fields and climate room. The stoma number of the plants grown in the fields was essentially higher, e.g. by more than 25% on the adaxial surface.

On SzD level a difference of 0.1% was observed in stoma number between the two surfaces, also, on the basis of the results related both to the climate room and the fields. In the case of both wheats the stoma number on the abxaial surface was lower: 86% (climate room) and 76% (fields), resp. of the amount detectable on the adaxial surface in GK Szeged; and 75% (climate room) and 73% (fields) in the case of Jubilejnaja 50.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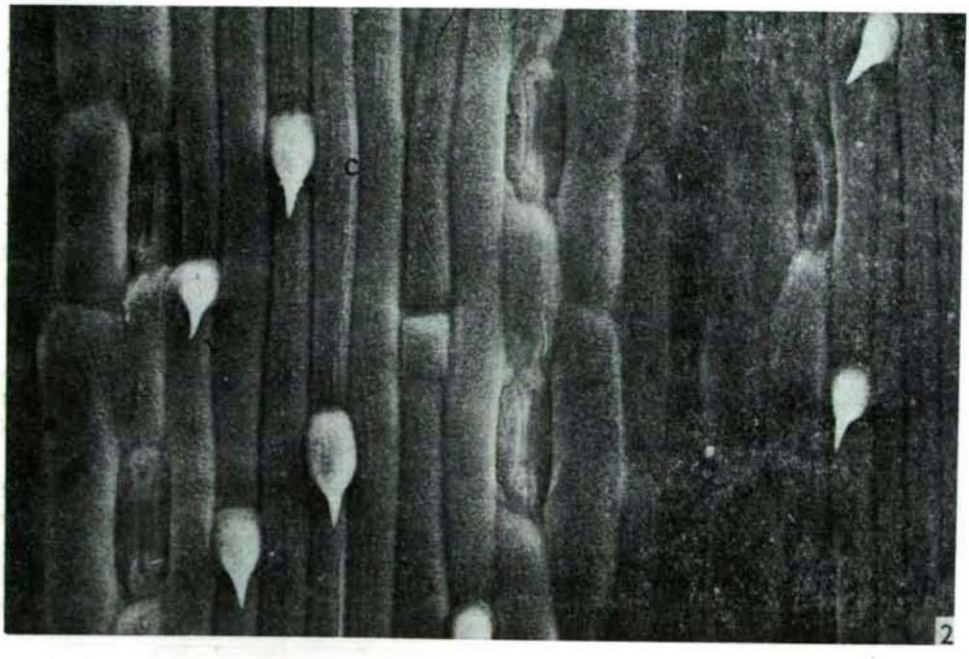
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AUSTIN et al. (1981) also obtained higher stoma number in mean values on the adaxial surfaces related to 15 wheat genotypes.

The frequency of stomata is always higher on the leaf surfaces than on the reverse sides in the *Triticum* species and types (TEARE et al., 1971) There are also significant differences between the two types on the basis of the stoma number on both surfaces. The stoma number of the GK Szeged cv. flag leaves is higher on both surfaces (Table 4).

On the flag leaves on the *Triticum* and *Aegilops* species the stoma incidence is in positive correlation with the maximal rate of photosynthesis (AUSTIN et al..1982). It is also expectable on the basis of the higher stoma amount of GK Szeged that the flag leaves bind more carbon dioxide related to unit surface, than the flag leaves Jubilejnaja 50 cv. The difference between the two surfaces is greater in trichome number. The amount of trichomes on the abaxial surface was only 42% (GK Szeged — climate room) and 45% (Jubilejnaja 50 — climate room), resp. of that on the adaxial surface (Table 4).

The measurements of the guard cells were compared on the basis of the length of two guard cells; as their joint width largely depends on the degree of openness of the stoma. On the adaxial side of the GK Szeged cv. flag leaves the average length of the guard cells was also greater besides greater stoma density (Table 4).

Great difference could be observed between the two surfaces in the amount of epicuticular wax. The adaxial surface of the flag leaves of both wheats was compactly covered by wax needles — except the protruding parts of the trichomes and the cuticle helix around the stoma (Plates III, IV., Picture 1). The density of the wax rods was greater on the adaxial surface of GK Szeged cv., than Jubilejnaja 50 cv. On the contrary, the wax needles were scarcely observed on the abaxial surface Plate V, Picture 1). The wax covering was not even on the adaxial surface, expressedly in the Jubilejnaja 50 cv. smaller-larger bare areas were detectable on this surface (Plates III, IV. Picture 1).

On the basis of the ratio of the surface wax coating and bare areas — in their studies on maize leaves — GÖRÖG et al. (1981) consider it to have determinant role in the permeation of herbicides and other substances through the surface.

STRUCTURE OF MESOPHYLL AND PHOTOSYNTHESIS

Since it has been proved that at high light intensity the flag leaves of diploid wheats have higher net photosynthesis rate than those of hexaploids (KHAN and TSUNODA, 1970; EVANS and DUNSTONA, 1970; GIFFORD and EVANS, 1973, AUSTIN et al., 1982), sereval authors interprent this also with the anatomical structure of the leaves (DUNSTONE and EVANS, 1974; PARKER and FORD, 1982; AUSTIN et al., 1982).

Those data (BONSTRA, 1937; SIMPSON, 1968; FOCKE, 1973; NALBORCZYK, 1978) according to which the flag leaves have significant role compared to other leaves in the formation of assimilates stored in the grains also raise the conception that there is a difference in the tissue structure of the flag and second leaves, too.

CHONAN (1965) demonstrated that the mesophyll cells of the upper leaves of wheat rea larger and more lobed than the lower ones. With the increase in lobe number the ratio

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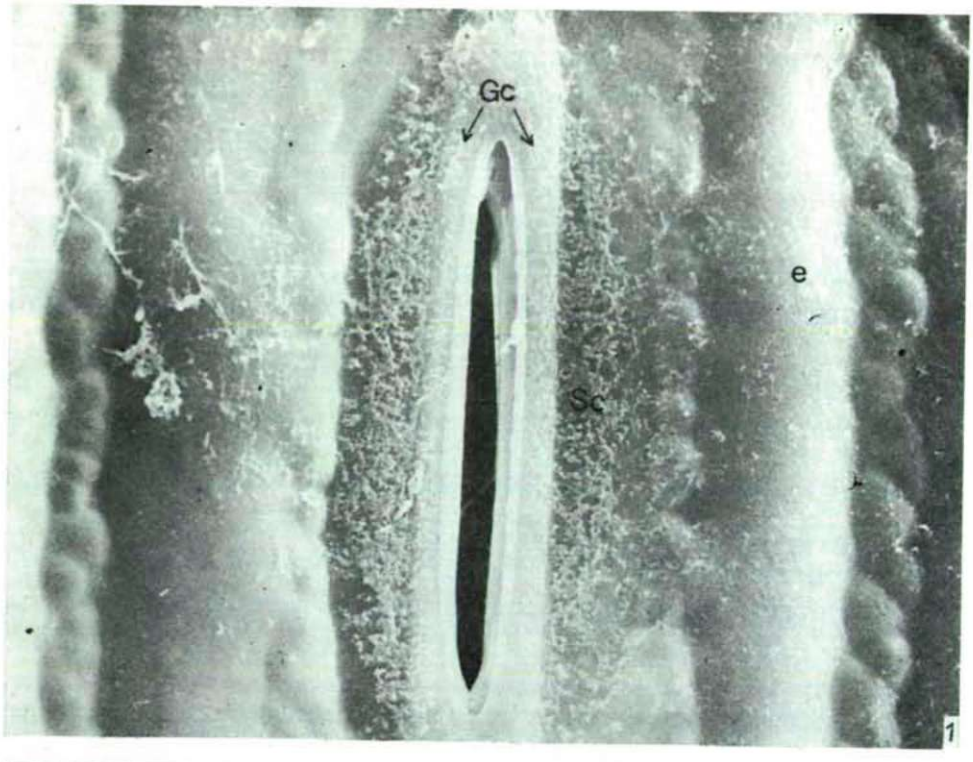
Plate IV. Adaxial surface of Jubilejnaja 50 flag leaf: Picture 1: Smaller-larger "bare areas" (ba) between the epicuticular wax needles (SEM, M: ~1250 x). Picture 2: Longitudinally running cuticle stripes (above the vein) (C) and the "bare areas" on the outer tangential wall of the epidermis cells. (M: ~300 x).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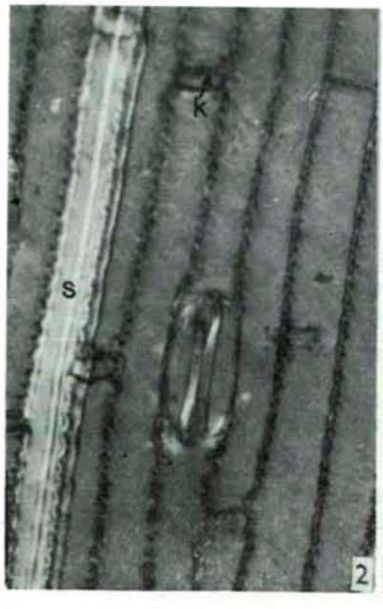
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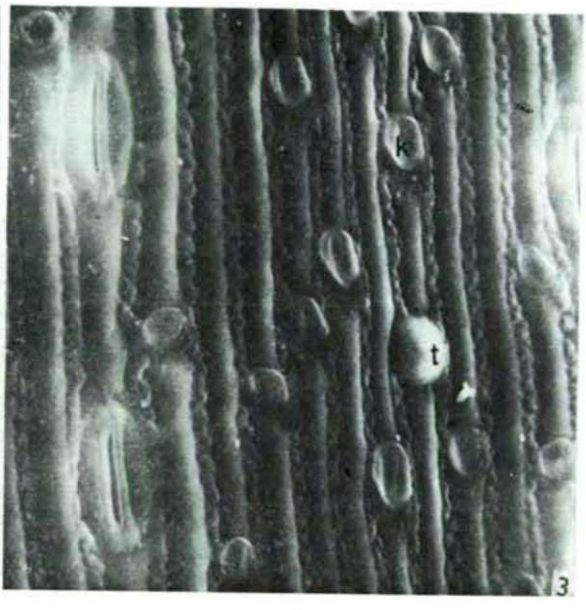
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of cell area per cell volume also increases, and this — according to our assumption-makes possible a more intensive gas exchange (binding of carbon dioxide).

The length and number of lobes of the mesophyll cells of the hexaploid *T. aestivum* cv. Professeur Marchal flag leaves are the double that of the diploid T urartu cells, but in contrast to the publication of CHONAN (1965) the cell area per cell volume ratio does not differ singificantly (PARKER and FORD, 1982). At the same time the net photosynthetic rate referring to the unit leaf area of the Professeur Marchal is 26 mg CO_2 $dm^{-2}h^{-1}$; being significantly lower than that of *T. urartu,* which is 43 mg CO_2 $dm^{-2}h^{-1}$ (AUSTIN et al., 1982).

From the point of view of CO_2 uptake PARKER and FORD (1982) consider rather the area of the air-filled intercellular space, and the ratio of internal exposed mesophyll cell area to external leaf area, resp. to be important; and not the whole mesophyll cell area. If this ratio increases, there is a decrease in the resistance in the diffusion of carbon dioxide into the mesophyll cells.

The intercellular surface per leaf area ratio is 15.3 in case of *T. urartu;* this is higher thau iu case of Professeur Marchal, where it is 10.5 (PARKER and FORD, 1982). Therefore, it is not in contrast with the variation of net photosynthesis in these two wheat types; according to our epinion, however, this explanation is not satisfactory.

Apart from the above, PARKER and FORD (1982) also bring the higher photosynthetic rate of *T. urartu* in connection with the fact that in the flag leaves of *T. urartu* the watetmovement of the photosynthates and between the veins and chloroplasts is possibly longer — due to the more dense situation of the veins — than in the case of P. Marchal, where there is a greater distance between the veins.

The afore-described notions are supported by experiments, nevertheless, in our opinion they are one-sided and there is still no satisfactory exlpanation to the integrated functioning of the mesophyll; the significance of the lobed structure of mesophyll cells; the cause of the changes in lobe number.

According to our assumption the lobed structure of the mesophyll cells has two essential significances: First, the cell volume is capable of increasing in such „quantum" manner (hexaploid wheats), that the cell area only decreases in a small degree, resp., the cell area per cell volume ratio does not change essentially. Second, the lobed structure makes possible a more effective adaptation to the differing light conditions. When the mesophyll cells under the epidermis become elongated, they become many-lobed and their height decreases (Table 3, Fig. 4). Thus, in one cell, close to the surface (light) there are several such chloroplasts which throw less shadow on each other and accommodate better to the high light intensity. In the mesophyll of flag leaves the ratio of longer and many-lobed mesophyll cells increases; that of the high and shorter cells decreases with the increase in density of the veins (related to the second leaves) (Fig. 4).

It has been demonstrated in our earlier publications (MARÓTI, 1976; MARÓTI and GÁBOR, 1976) that in the palisade chloroplasts, the amounts of chlorophyll-b, neoxanthine, and lutein characteristic of the II. photochemical system are lower, and the area of adhered membranes (grana thylakoids) is smaller than in the spongy parenchymal

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Plate V. Picture 1: Scarce wax-coating on the abaxial surface of GK Szeged flag leaf (SEM, M: ~ 1250 x) Gc = guard cell, Sc = subsidiary cell, e = epidermis cell. Picture 2: Polarization light microscopic picture of the narrow epidermis cells of the abaxial surface, above the stoma-free sclerenchyma (s). GK Szeged second leaf, M: 300 x) K = silica cell. Picture 3: SEM picture of the sections of stoma-lined, wide epidermis cells and those densely covered by stoma-free trichomes (t), silica cells (K). (M: ~300 x).

chloroplasts. It is also known from the study of SKENE (1974) that the individual lamella (granum ratio increases significantly in the palisade cell chloroplasts. Furthermore, the „palisade character" increases in the flag leaves.

On the basis of the above, it is assumed that in the chloroplasts of wheat flag leaves also (compared to the second leaves), there is a higher ratio of stoma lamellae, and cyclic photophosphorylation providable to this membrane, resp. (and relatively independent of the linear electron transport). In case of high light intensity therefore, the chloroplasts of flag leaves produce more ATP than those of the lower leaves, and this enables enhanced triosphosphate transport from the chloroplasts, and saccharose transport from the cytoplasm.

It the above hypothesis is applied to the two wheat types, the result is that Jubilejnaja 50 cv is a species accommodating better to high light intensity; but its net photosynthetic rate may be lower; its cyclic photophosphorylation more ample than in case of GK Szeged cv.

Nevertheless, further and many-sided experiments are necessary to become familiar with the facts.

Acknowledgement

We should like to express our sincere thanks to Professor **Péter Kása,** director of the Central Laboratory of the Medical University of Szeged, for enabling the scanning electron microscopic examinations, and also to **Zoltán Farkas,** technician, for contributing to the preparations of the material and the photographs.

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Address of the authors:
DR. SZ. PATAKY
DR. I. MARÓTI
Department of Botany, A. J. University
H-6701 Szeged, P.O. Box 657, Hungary
DR. J. BÁLINT
Cereal Research Institute
H-6726 Szeged, Hungary

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SCANNING ELECTRON MICROSCOPY OF SOME SELECTED RECENT AMENTIFLORAE POLLENS II.

M. KEDVES and Á. PÁRDUTZ

Department of Botany, Attila József University, and Institute of Biophysics, Biological Research Center of the Hungarian Academy of Science, Szeged
(Received September 3, 1981)

Abstract

From the *Myrica*, and *Gale* genus one species, from the genus *Betula* 17 species were investigated by the scanning electron microscope, from the latter mentioned genus two hybrid species, too. Based on recent data, compared with the earlier SEM data of the brevaxonate *Amentiflorae* pollen grains *(Corylus, Carpinus, Ostrya, Casuarina)* we may conclude the following: 1. The submicroscopic characteristic feature of the brevaxonate *Amentiflorae* pollen grains may be characterized by the coni. In each case the basis diameter and the number per square micron have taxonomic value. 2. Inside the submicroscopic coni, two types may be distinguished; a) simple, when these ornamental elements are prominent from the smooth tectum, b) composite, in this case the coni are on short striae. 3. The two kinds of coni have intergeneric and intrageneric taxonomic value. 4. The prominent germinal area is in general connected by submicroscopic striae.
Key words: Palynology, recent, Amentiflorae, SEM.

Introduction

In the evolutionary point of view of the *Angiospermatophyte*, the *Amentiflorae* have a peculiar importance. Concerning this question PRAGLOWSKI (1962, p. 46) wrote the following: „I have added a few lines on some exotic amentaceous pollen types. They may perhaps be of interest to taxonomists and to paleobotanists dealing, for example, with the pollen flora of Upper Cretaceous." In a previous paper (KEDVES, 1979) the SEM results of the following genuses were summarized: *Corylus, Ostrya, Carpinus, Casuarina.* This will be completed by the present paper, with the results of the *Myrica, Gale* and *Betula* genus. In this way, if not in all details, but we will have a general knowledge about this question.

Materials and Methods

Essentially the same published previously with the difference that the pictures were taken in the Electron Microscope Laboratory of the Institute of Biophysics of the Biological Center of the Hungarian Academy of Science on a JEOL-100B JEM-ASID scanning supplement.

Results

Similarly to the previous publication, the most important morphologic, primarily SEM characteristic features are summarized in a table.

Species	Coni basis	No. coni per μm^2	Stries	Diameter, μm	Prominent germ.
		Myrica			
M. cerifera	0.3—0.35	3—4	—	25—32	±
		Gale			
G. palustris	0.3—0.4	2—3	—	22—28	±
		Betula			
B. verrucosa	0.2—0.4	2—3	+	22—26	±
B. albo-sinensis	0.2—0.3	4—5	±	28—37	+
B. nana	0.2—0.3	2—3	±	20—27	+
B. nigra	0.2—0.3	2—3	±	27—34	+
B. pendula	0.2—0.3	3—4	+	25—30	+
B. chinensis	0.2—0.3	3—4	±	34—37	±
B. papyrifera	0.15—0.2	2—3	+	30—36	±
B. pubescens carpathica	0.2—0.3	4—5	+	27—35	±
B. grossa	0.2—0.3	3—5	+	25—32	±
B. mandschurica	0.2—0.3	2—3	±	24—28	+
B. ermani	0.2—0.3	3—4	+	32—44	±
B. davurica	0.15—0.2	2—3	+	27—37	±
B. lenta	0.2—0.3	2—3	±	25—32	±
B. pubescens urticifolia	0.2—0.3	2—3	+	32—43	±
B. humilis	0.3—0.35	3—4	+	19—24	±
B. x aurata	0.15—0.2	3—4	+	27—32	±
B. x intermedia	0.2—0.3	3—4	+	25—32	—

The most important results are as follows:

The surface ornamentation of the species investigated from the *Myrica* and *Gale* genuses alike are simple coni, without short striae. There is no significant difference in the diameter of the coni basis, but the number of coni per square micron is different. But it must be emphasized that these characteristic features have their variation, so this evaluation must be taken into consideration with criticism.

The *Betula* genus is heterogeneous in palynological point of view, but as common characteristic feature the submicroscopic coni on short striae may be emphasized.

On the basis of the coni number per square micron the following groups may be established:

1.1, 2—3 — *B. verrucosa*
B. nana
B. nigra
B. papyrifera
B. mandschurica
B. davurica
B. lenta
B. pubescens urticifolia

1.2, 3—4—*B. pendula*
B. chinensis
B. ermani
B. humilis
B. x aurata
B. x intermedia
1.3, 3—5—*B. grossa*
1.4, 4—5—*B. albo-sinensis*
B. pubescens carpathica

It is worth mentioning that the two examined subspecies of *B. pubescens* are different in this point of view.

When we take for basis the diameter of the coni, the sequences are as follows:

2.1, 0.15—0.2 — *B. papyrifera*
B. davurica
B. x armata
2.2, 0.20—0.3 — *B. albo-sinensis*
B. nana
B. nigra
B. pendula
B. chinensis
B. pubescens carpathica
B. grossa
B. mandschurica
B. ermani
B. lenta
B. pubescens urticifolia
B. x intermedia
2.3, 0.2—0.4 — *B. verrucosa*
2.4, 0.3—0.35 — *B. humilis*

Noteworthy, the two subspecies of *B. pubescens* are identic, but the two hybrids which where identic in the previous sequence, in this case are different.

Taking into consideration the two characters, the following species are in the same group:

1.1×2.1 *B. papyrifera*
B. davurica
1.1×2.2 *B. nana*
B. nigra
B. mandschurica
B. lenta
B. pubescens urticifolia
1.1×2.3 *B. verrucosa*
1.2×2.1 *B. aurata*
1.2×2.2 *B. pendula*
B. chinensis
B. ermani
B. x intermedia
1.2×2.4 *B. humilis*
1.3×2.2 *B. grossa*
1.4×2.2 *B. albo-sinensis*
B. pubescens carpathica

But when we compare the light microscopic morphological characteristic features with the SEM characters, the conclusions are not unianimous. Of these the following may be emphasized:

1. The pollen grains of *B. humilis* in comparison to the other studied species are relatively small in size, and the total SEM characteristic features are also different. *B. grossa* separates well by its SEM characteristics, but its size is average. The two species with the greater size *(B. ermani, B. pubescens urticifolia)* have different SEM sculpture except the diameter of the coni basis.

2. Regarding the germinal area, the hybrid *B. x intermedia* may be accentuated because between the examined species only in this case the germinal area was not prominent, but the total SEM characteristics are not different from the others. Based on our present knowledge, the prominent germinal area is generally connected with more or less characteristic striae, except the *B. pendula, B. chinensis* and *B. lenta.*

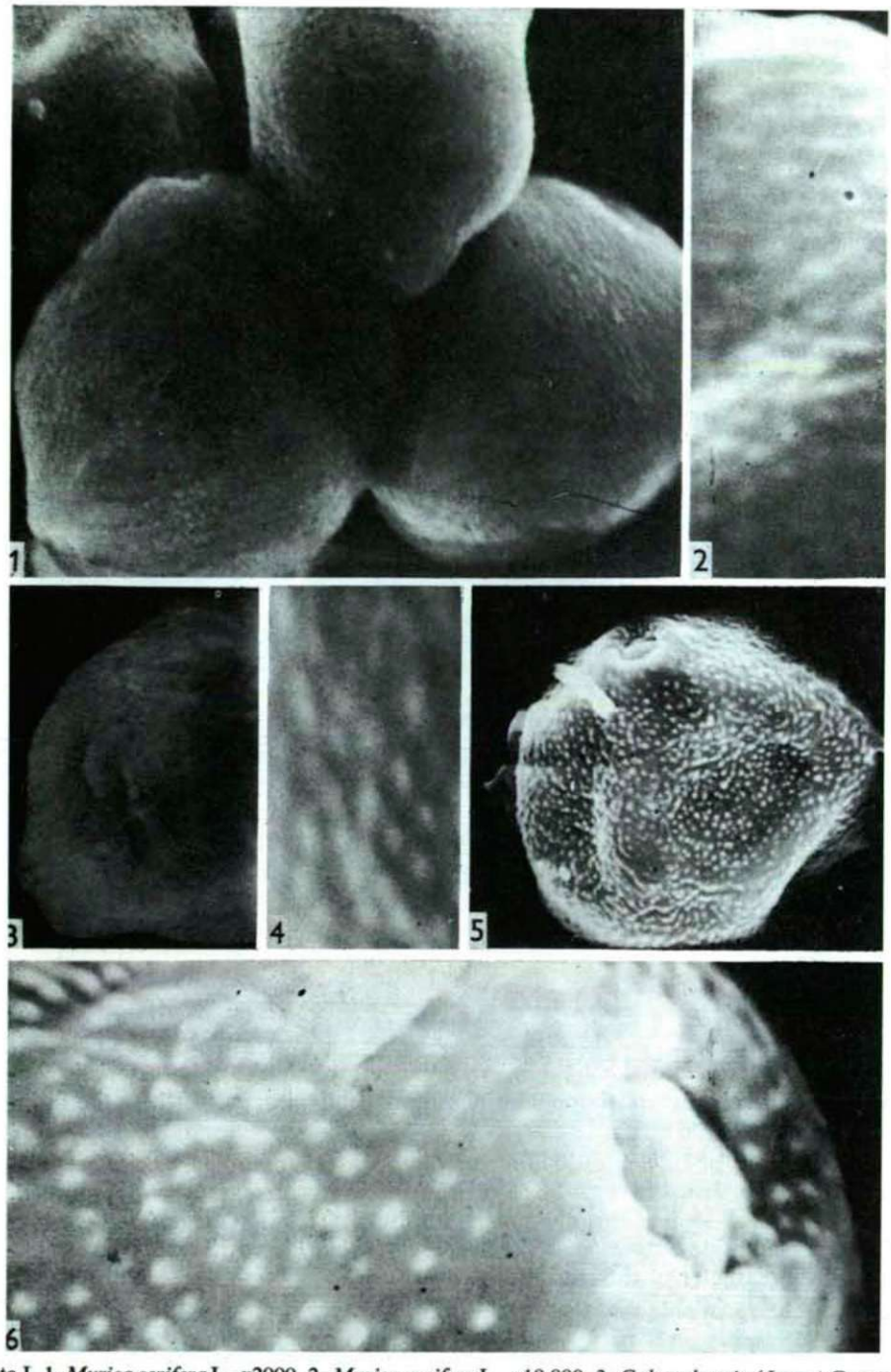

Plate I. 1. *Myrica cerifera* L., x2000. 2. *Myrica cerifera* L., x10 000. 3. *Gale palustris (Lam.)* CHEVAL., x2000. 4. *Gale palustris (Lam.)* CHEVAL., x10 000. 5. *Betula verrucosa* EHRH., x2000. 6. *Betula verrucosa* EHRH., x10 000.

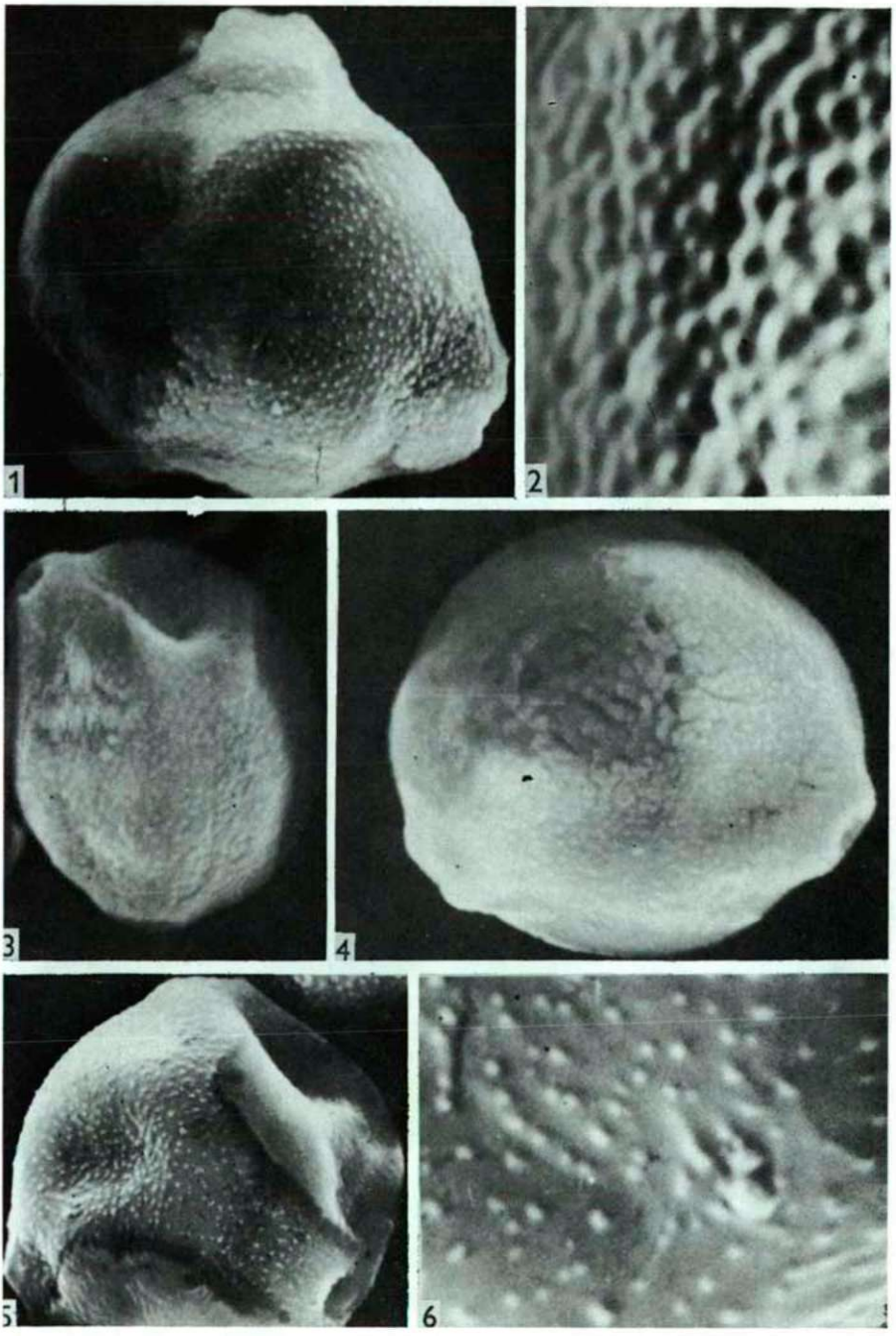

Plate II. 1. *Betula albo-sinensis* BURK., x2000. 2. *Betula albo-sinensis* BURK., x10 000. 3. *Betula nana* L., x2000. 4. *Betula nigra* L., x2000. 5. *Betula pendula* RORTH., x2000. 6. *Betula pendula* RORTH., x10 000.

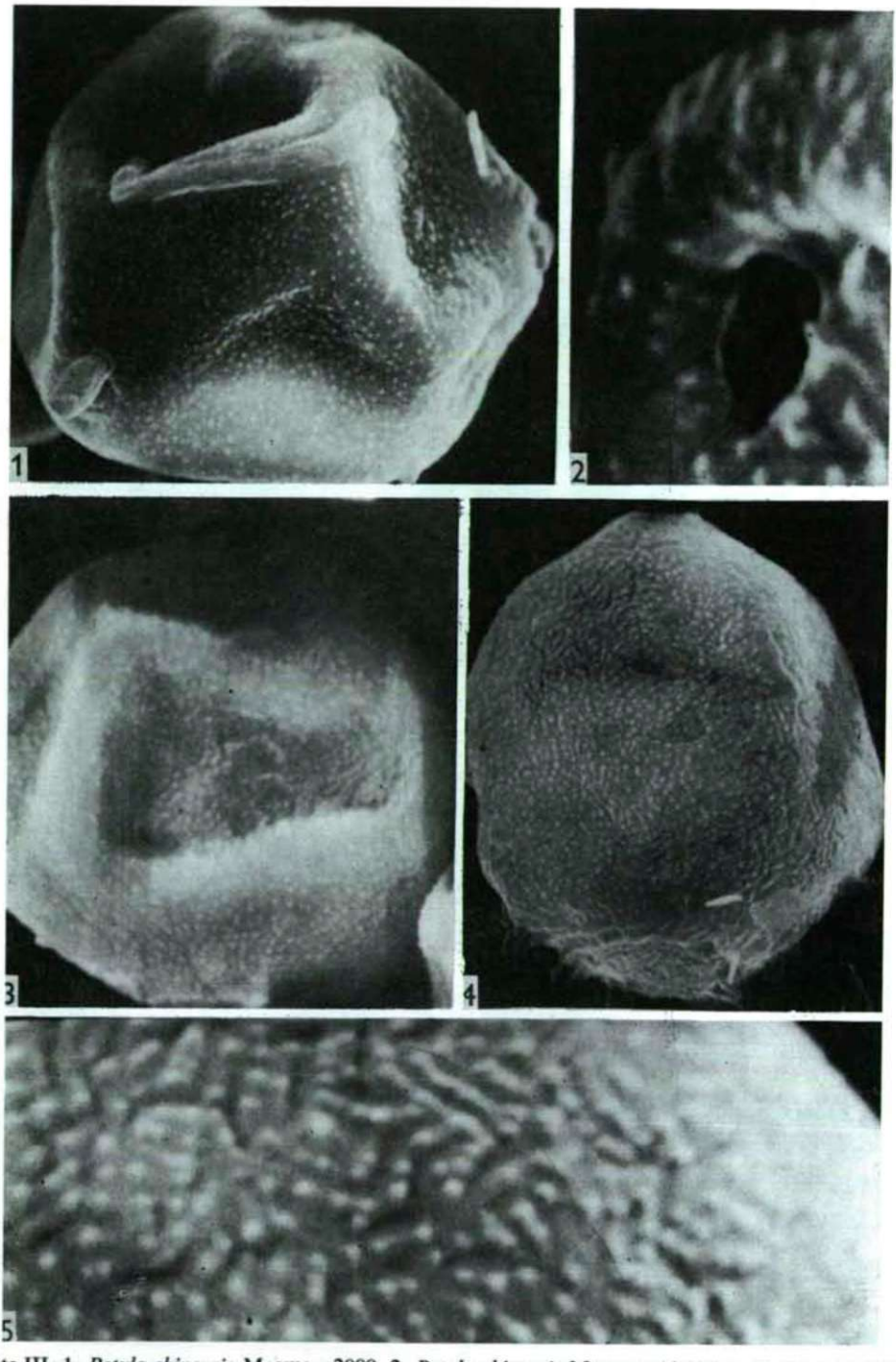

Plate III. 1. *Betula chinensis* MAXIM, x2000. 2. *Betula chinensis* MAXIM, x10 000. 3. *Betula papyrifera* MARSH, x2000. 4. *Betula pubescens* EHRH. *subsp. carpathica* (WILLD.) ASCHERSON et GRAEBNER, x2000. 5. *Betula pubescens* EHRH. *subsp. carpathica* (WILLD.) ASCHERSON et GRAEBNER, x10 000.

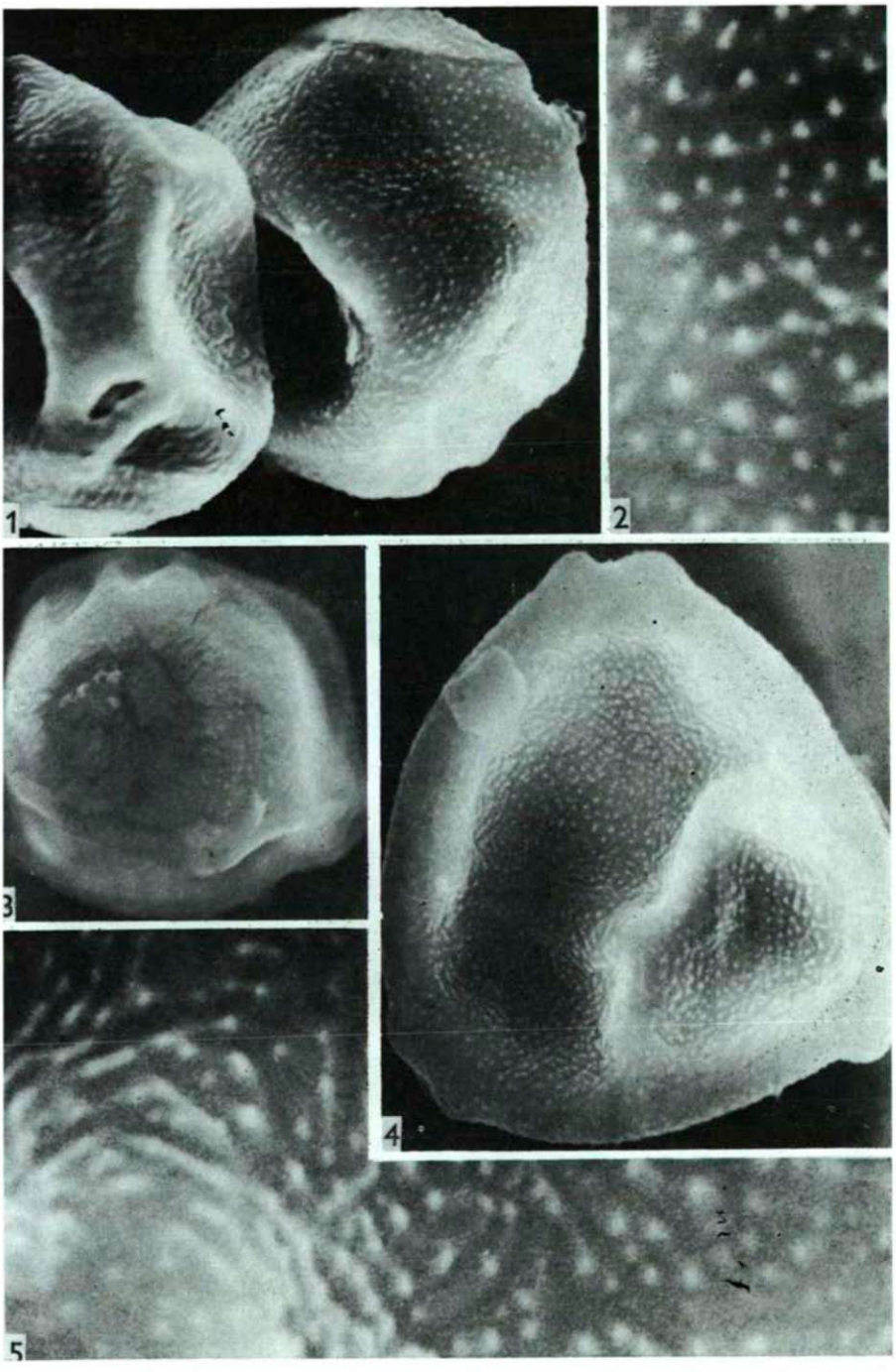

Plate IV. 1. *Betula grossa* SIEB. et ZUCC., x2000. 2. *Betula grossa* SIEB. et ZUCC.,x10 000. 3. *Betula mandschurica (Regel)* NAKAI, x2000. 4. *Betula ermani* CHAM., x2000. 5. *Betula ermani* CHAM., x10 000.

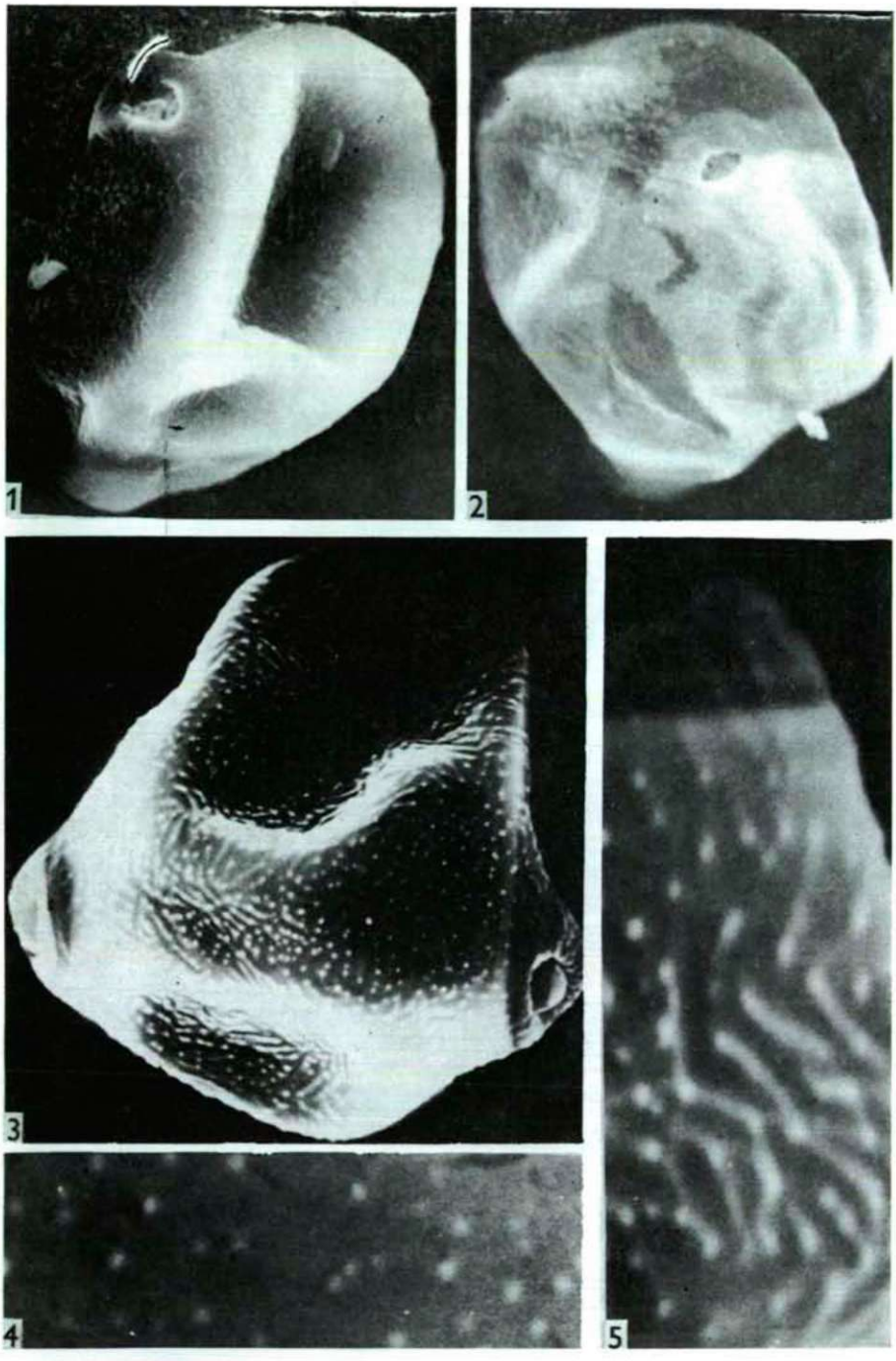

Plate V. 1. *Betula davurica* PALL., x2000. 2. *Betula lenta* L., x2000. 3. *Betula pubescens* EHRH. *var. urticifolia* REGEL, x2000. 4. *Betula pubescens* EHRH. *var. urticifolia* REGEL, x10 000. 5. *Betula pubescens* EHRH. *var. urticifolia* REGEL, x10 000.

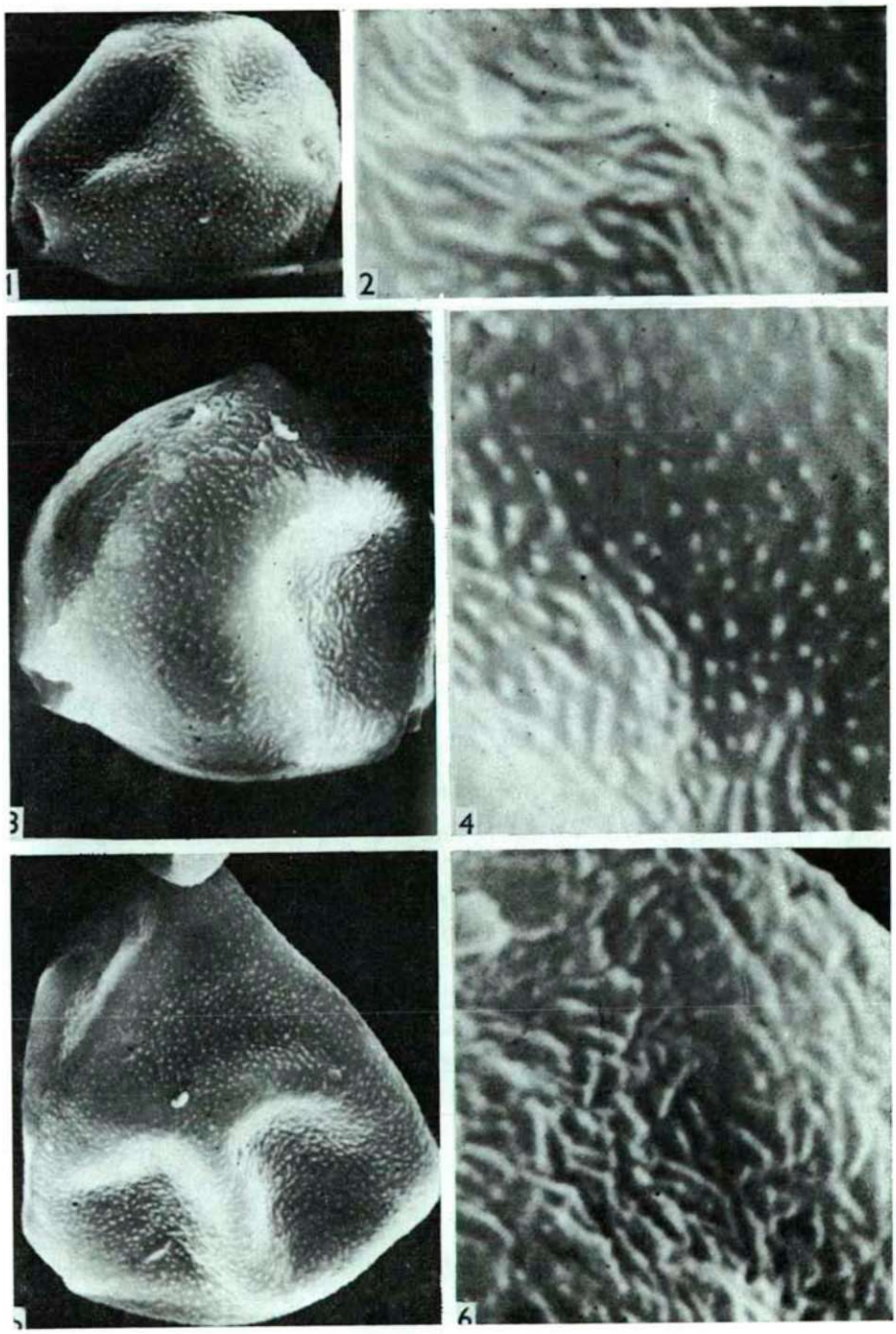

Plate VI. 1. *Betula humilis* SCHRANK, x2000. 2. *Betula humilis* SCHRANK, x10 000. 3. *Betula x aurata* BORKH., x2000. 4. *Betula x aurata* BORKH., x10 000. 5. *Betula x intermedia* THOMAS ex REICHB., x2000. 6. *Betula x intermedia* THOMAS ex REICHB., x10 000.

Discussions

Based on our present data and completed with those previously published, it may be concluded that on the taxonomic or other evaluation of the pollen morphological characteristic features it is necessary to be careful because some previously con-

cepts taken as general need to be modified. So the striae (suprategillar crest; PRAGLOWSKI, 1962, ridges, TAKEOKA and STIX, 1963) are not exclusively the characteristic features of the *Betula* genus, this may occur in other genuses too, for example, *Ostrya, Carpinus, Corylus* and *Casuarina* pro parte. The relations between the TEM and SEM data are not always unianimous either. In this point of view as primitive example the *Juglandaceae* and *Myricaceae* may be emphasized, where the simple coni is connected with granular infratectum. Similarly the rather peculiar infratectum is also suitable for taxonomic and evolutionary evaluations. The present results also support the necessity of the electron microscope investigations for more exact palynological evaluations.

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Address of the authors:
DR. M. KEDVES
Department of Botany A. J. University
H-6701 Szeged, P. O. Box 657, Hungary
DR. Á. PÁRDUTZ
Institute of Biophysics,
Biological Research Center of the
Hungarian Academy of Science
H-6701 Szeged, Hungary

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STUDIES ON THE POLLEN GRAINS OF RECENT CASTANEOIDEAE. II

M. KEDVES and Á. PÁRDUTZ

Department of Botany, Attila József University, and Institute of Biophysics, Biological Research Center of the Hungarian Academy of Science, Szeged
(Received September 3, 1981)

Abstract

During our transmission electron microscope investigations on recent *Castaneoideae* taxa, Ubish bodies, extratapetal membrane, and pellicula was observed outer the pollen grains. The exine of all the investigated species is tectate and perforated with channels. The infratectal layer is columellar and beneath the foot layer there is a granular endexine, in each case lamellar in the apertural area. In the apertural area, the ectexine is thinner than extragerminally. The contour of the cavern is the consequence of the refraction of light of the cavity between the ectexine and endexine. The general aspect of the ultrastructure of the ectexine and the endexine is identical with those of the earlier described extant pollen grain of *Castanea* type. Proved by the SEM data, the surface of the pollen grains of the *Castaneoideae* taxa is not psilate, but ornamented with short striae. This characteristic feature separates well from the brevaxonate *Amentiflorae* pollen grains, which are ornamented by characteristic coni.
Key words: Palynology, recent, Castaneoideae, TEM and SEM.

Introduction

In an earlier paper (KEDVES, 1982), the light microscope results of the *Castaneoideae* pollen grains are presented. As it was pointed out in this paper, the light microscope data should be completed by electron microscope results. This is the aim of this paper.

Previous TEM and SEM data were published by TAKEOKA (1965), MARTIN and DREW (1969), HESSE (1978), CREPET and DAGHLIAN (1980) and LIEUX (1980). TEM data of dispersed extant pollen grains were published by KEDVES and PÁRDUTZ (1973) and CREPET and DAGHLIAN (1980).

Materials and Methods

The material for our investigations was described in the previous paper. For TEM studies, acetolyzed and non-acetolyzed pollen grains were used, and were prepared with OsO_4. For SEM investigations non-acetolyzed pollen grains were mounted on polyvinylchlorid adhesive material then covered by gold-palladium. The pictures were taken in the EM Laboratory of the Institute of Biophysics, Biological Research Center of the Hungarian Academy of Science on a JEM-ASID scanning supplement of a JEOL-100B electron microscope.

Results

1. Transmission electron microscope results

1. *Castanea americana* RAF. (Plate I, Figs. 1, 2)

Interapertural exine. — The exine consists of ectexine and granular endexine. The ectexine is tectate, perforate, much thicker than the endexine. The three layers of the

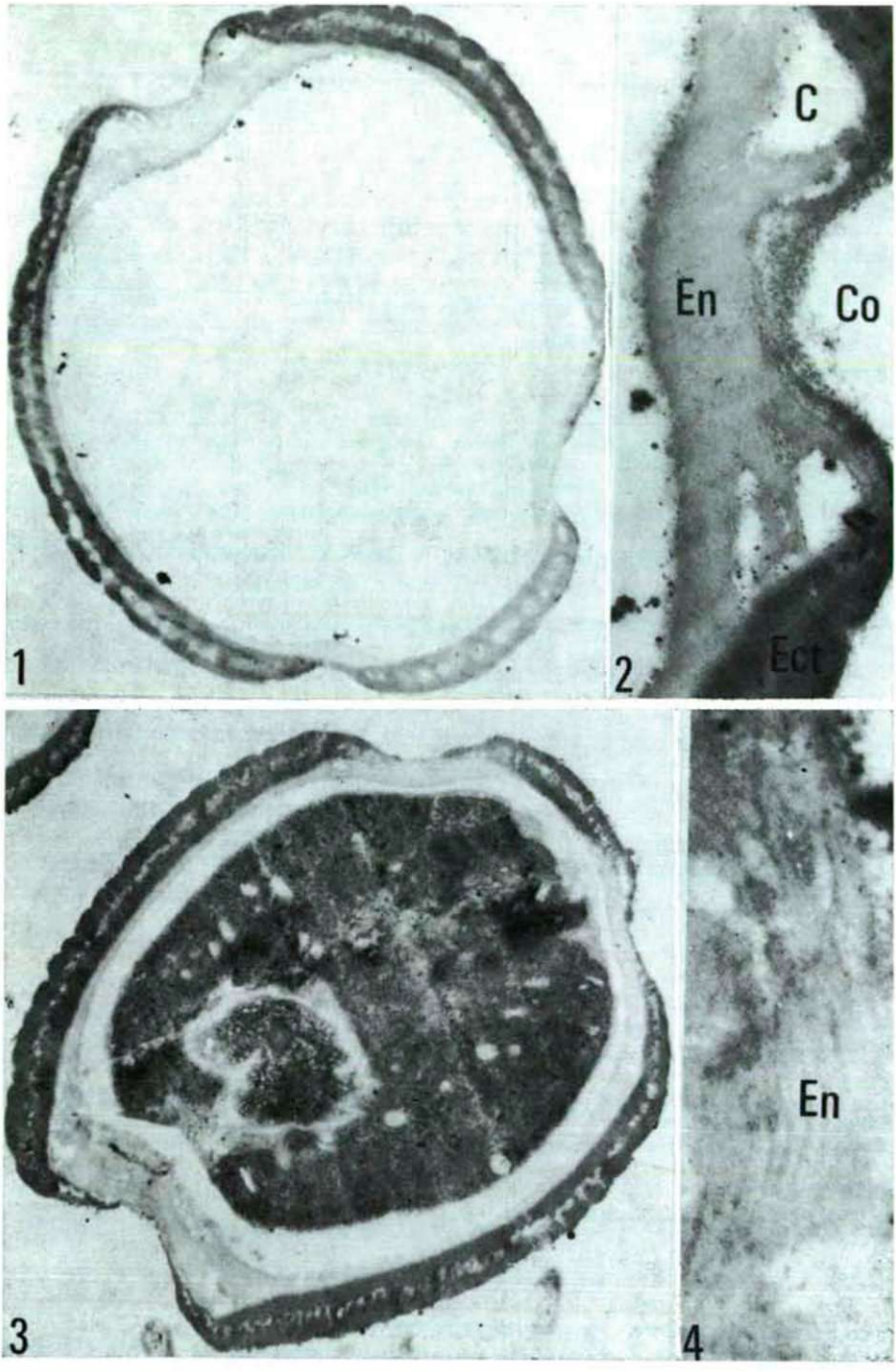

Plate I. 1. *Castanea americana* RAF., cross section of acetolyzed pollen grain, x10 000.
2. *Castanea americana* RAF., ultrastructure of the apertural area, x20 000.
3. *Castanea sativa* MILL., non-acetolyzed pollen grain, cross section, x10 000.
4. *Castanea sativa* MILL., lamellar endexine in the apertural area, x40 000.
C = cavern, Co = colpus, Ect = ectexine, En = endexine.

ectexine (tectum, infratectum and foot layer) are equal. The infratectal layer is columellar.

Apertural area. — Near the furrows, the ectexine is extremely thin, and the endexine is thick. In the colpal and the colporal area, the ectexine is granular (Plate I, Fig. 2, Co), and without threefold structure. The endexine becomes thick here, the ectexine protrudes out, and between the ect- and endexine there is a cavity (C).

2. *Castanea sativa* MILL. (Plate I, Figs. 3, 4, Plate II, Fig. 1)

Interapertural exine. — It consists of ectexine and endexine. The ectexine is tectate and channeled. The tectum and the foot layer are of equal thickness, the infratectum is a little thinner than the previously mentioned two layers. The fine structure of the infratectum is columellar. The endexine is granular, its thickness corresponds roughly to that of the infratectal layer.

Apertural area. — In the region of the furrows the ectexine becomes thinner. This thinning is uneven, the inner layers become thinner or disappear than the tectum. The endexine is much thicker than in the interapertural area. The cavern near the colpori forms as described previously, the ectexine becomes mostly a granular, not pluri-layered wall. In comparison to *C. americana* the lamellar ultrastructure of the endexine in the apertural area is worth mentioning (cf. NABLI, 1979).

3. *Castanopsis argyrophylla* KING (Plate II, Fig. 2)

Interapertural exine. — It consists of ectexine and granular endexine. The tectum in some places channeled. The infratectal layer is columellar, the three layers of the ectexine are equal.

Apertural area. — The ectexine near the furrows becomes extremely thin. The three ectexinal layers may not be recognized in this thin part. But the endexine is thick in this area. Near the colpori the ectexine is essentially identical with those of the colpi, but the thin more or less homogeneous part is a little longer. The cavern is essentially identical with the previously discussed ones but in this case the number of the cavities may be more than one. In the germinal area the ultrastructure of the endexine is granular.

4. *Castanopsis indica* DC. (Plate II, Fig. 3)

Interapertural exine. — It consists of ectexine and granular endexine. The ectexine is tectate, and based on our observations imperforate. Between the three ectexine layers the tectum is the thickest, the infratectal layer and the foot layer are equal. The infratectum is columellar.

Apertural area. — Near the furrows the ectexine become thinner, first of all the tectum, the endexine is only a little thicker than in the interapertural area. The apertural ectexine of the colpori is homogeneous, the endexine is granular, without lamellation. The cavern consists generally of one large and more smaller cavities.

5. *Castanopsis longispicata* HU (Plate II, Fig. 4, Plate III, Figs. 1—4)

At this species the ultrastructure of the elements which enclose the pollen grains was also suitable for investigations (Plate II, Fig. 4, Plate III, Fig. 1). Concerning the identification of the non exinous ultrastructural elements we are deeply indebted to Mr. PROF. DR. J. R. ROWLEY (Stockholms Universitet, Botaniska institutionen, Stockholm). We have observed numerous Ubish bodies, based on the literature data, its shape alters in the course of its growth. In our material we cannot observe these different forms, the bodies figured in this paper are probably the mature forms. As regards the origin of the Ubish bodies, see the papers of ROWLEY and ERDTMAN (1967) and ROWLEY and SKVARLA (1974). Based on the latter mentioned paper, the peculiar form of the Ubish bodies is determined by the glycocalyx of the plasma mem-

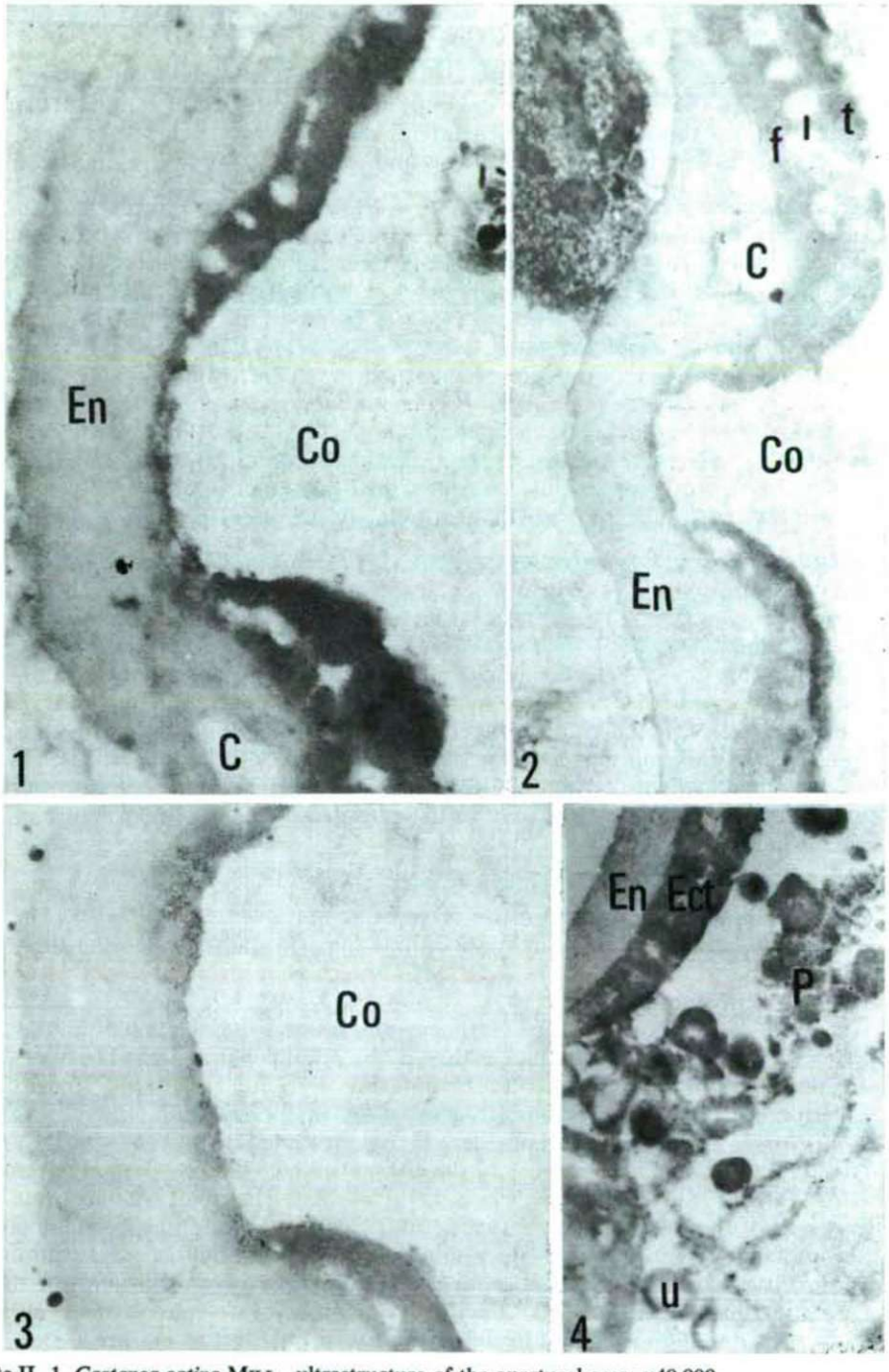

Plate II. 1. *Castanea sativa* MILL., ultrastructure of the apertural area, x40 000.
2. *Castanopsis argyrophylla* KING, ultrastructure of the apertural area, x20 000.
3. *Castanopsis indica* DC., ultrastructure of the apertural area, x40 000.
4. *Castanopsis longispicata* HU, ultrastructure of the exine and of the elements which enclose the pollen grains, x20 000.
C = cavern, Co = colpus, Ect = ectexine, t = tectum, i = infratectum, f = foot layer, En = endexine, P = pellicule U = Ubish bodies.

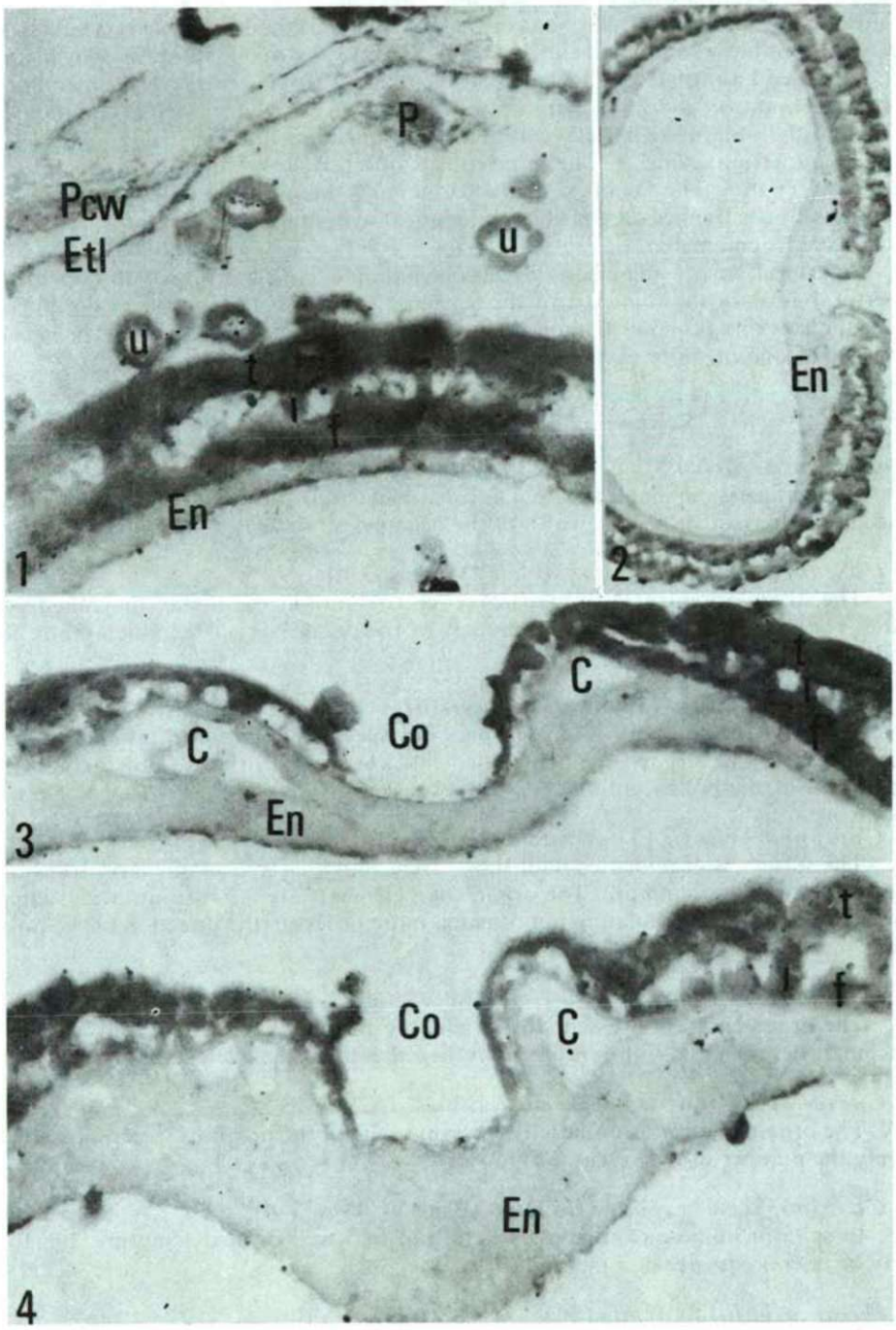

Plate III. 1. *Castanopsis longispicata* Hu, ultrastructure of the exine and of the elements which enclose the pollen grains. x20000.
2. *Castanopsis longispicata* Hu, longitudinal section of acetolyzed pollen grain, x10 000.
3, 4. *Castanopsis longispicata* Hu, ultrastructure of the apertural area, x20 000.
C = cavern, Co = colpus, t = tectum, i = infratectum, f = foot layer, En = endexine, Etl = extra tapetal lamellation, P = pellicule, Pcw = parietal cell wall, U = Ubish bodies.

brane. In connection with the question of its function the following papers are worth mentioning: ECHLIN (1971), DUNBAR (1973), VASIL (1973) and MASCARENHAS (1975). Its taxonomic value was pointed out by UENO (1959). Moreover, residues of pellicules (cf. CERCEAU-LARRIVAL and ROLAND-HEYDACKER, 1976) = extratapetal membrane (HESLOP-HARRISON, 1969) = extratapetal lamellation (ROWLEY, a letter communication) and the wall of the parietal cell occured in our ultra-thin sections.

Interapertural exine. — The exine consits of ectexine and granular endexine, this latter is very thin. The thickness of the tectum and the foot layer is equal, the infratectum is a little thinner, its thickness is identical with that of the endexine. The infratectal layer is columellar.

Apertural area. — The ectexine becomes thinner, the endexine is thicker in the apertural area. In the thin part of the germinal exine first the foot layer disappears after the ectexine becomes homogeneous, and near the endopori divide. The cavern consists of one or more cavities.

2. Scanning electron microscope results

1. *Castanea sativa* MILL. (Plate IV, Figs. 1, 2)

The surface is very finely striate. The ornamental elements are diagonally oriented, and bifurcate frequently. The width of the ornamentation is 0.1—0.2 μm.

2. *Castanea americana* RAF. (Plate IV, Figs. 3, 4)

The striate ornamentation may be recognized only in the highly magnified pictures. The short striae are oriented obliquely of the polar axis of the pollen grain, the width is about 0.2 μm.

3. *Castanea pumila* MILL. var. *angustifolia* (Plate IV, Figs. 5, 6)

Similarly to the above discussed previous species, the ornamentation is not so characteristic. The striate ornamental elements are likevise oriented obliquely to the polar axis of the pollen grain.

4. *Castanea evansii* ELM. (Plate V, Figs. 1, 2)

The surface is ornamented with short striae, but this is in some places transitional to the rugulate sculpture. The ornamental elements are 0.2—0.3 μm wide, sometimes bifurcate, and undulating but oriented more or less in the direction of the polar axis.

5. *Castanopsis longispicata* HU (Plate V, Figs. 3, 4)

The ornamentation is essentially striate, the elements are 0.2—0.3 μm wide and irregular. Between this sculpture elements there are often small verrucae.

6. *Castanopsis indica* DC. (Plate VI, Figs. 1, 2)

The ornamentation is essentially the same as with the previous species, but relatively the number of the verrucae is lower.

7. *Castanopsis argyrophylla* KING (Plate VI, Figs. 3, 4)

In spite of numerous attempts we did not find well defined sculpture, but this may be in consequence of a methodical error.

8. *Pasania calathiformis* (SKAN.) H. et C. (Plate VII, Figs, 1, 2)

The surface is rugulate, foveolate, and only rarely striate, the width of the ornamental elements is 0.3—0.4 μm.

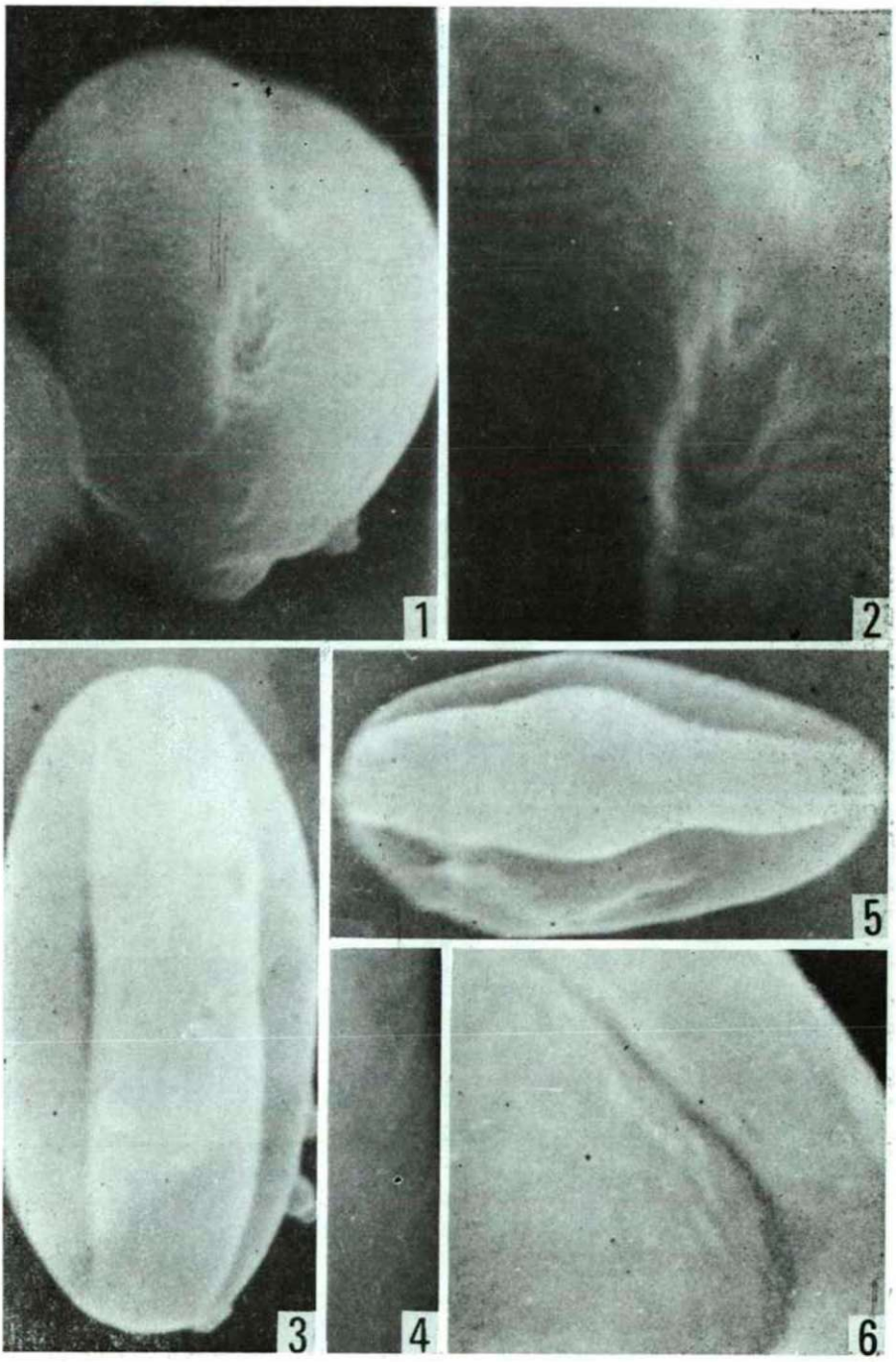

Plate IV. 1. *Castanea sativa* MILL., x5000.
2. *Castanea sativa* MILL., x10 000.
3. *Castanea americana* RAF., x5000.
4. *Castanea americana* RAF., x10 000.
5. *Castanea pumila* MILL. var. *angustifolia*, x5000.
6. *Castanea pumila* MILL. var. *angustifolia*, x10 000.

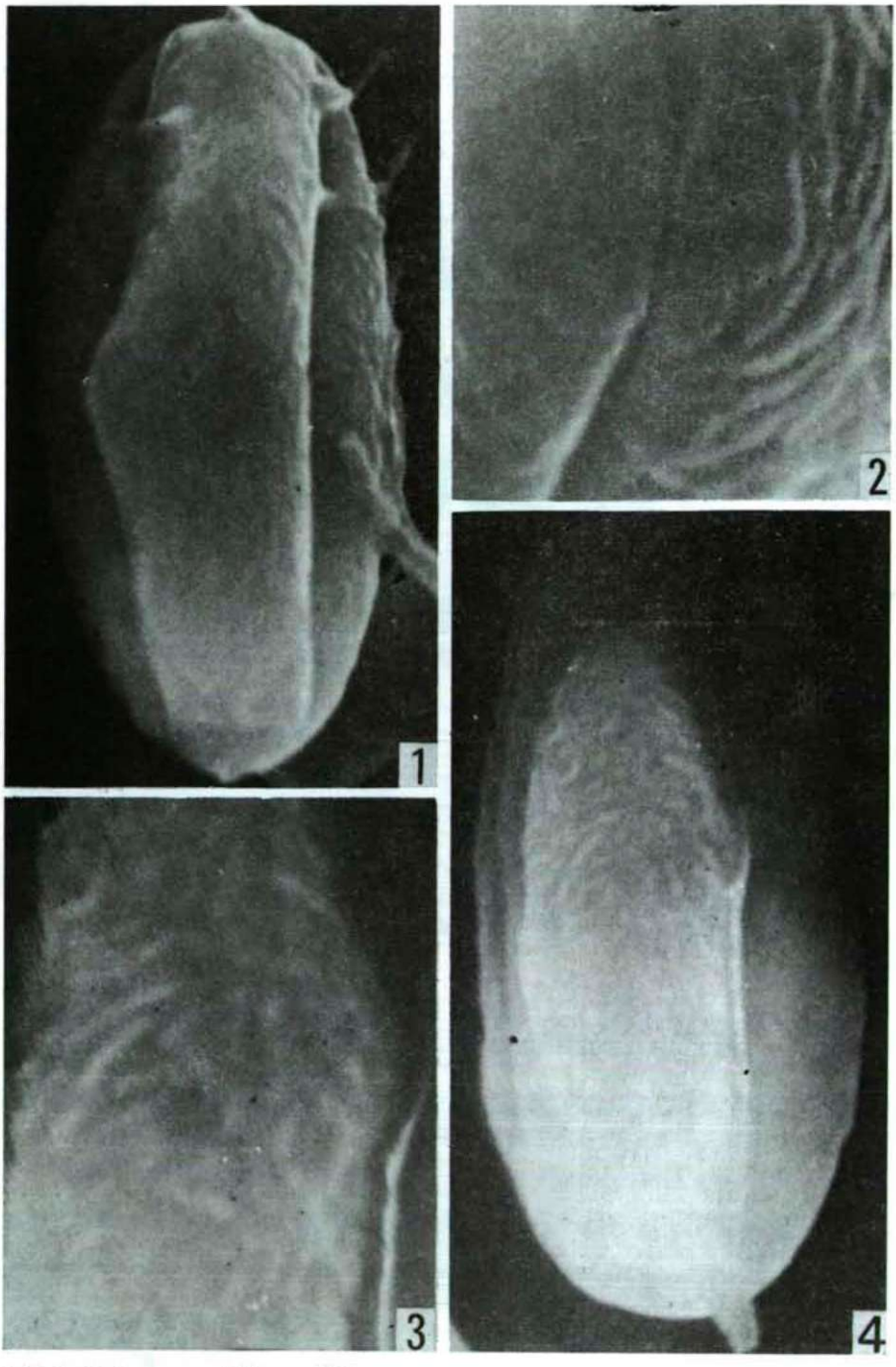

Plate V. 1. *Castanea evansii* ELM., x5000.
2. *Castanea evansii* ELM., x10 000.
3. *Castanopsis longispicata* HU, x10 000.
4. *Castanopsis longispicata* HU, x5000.

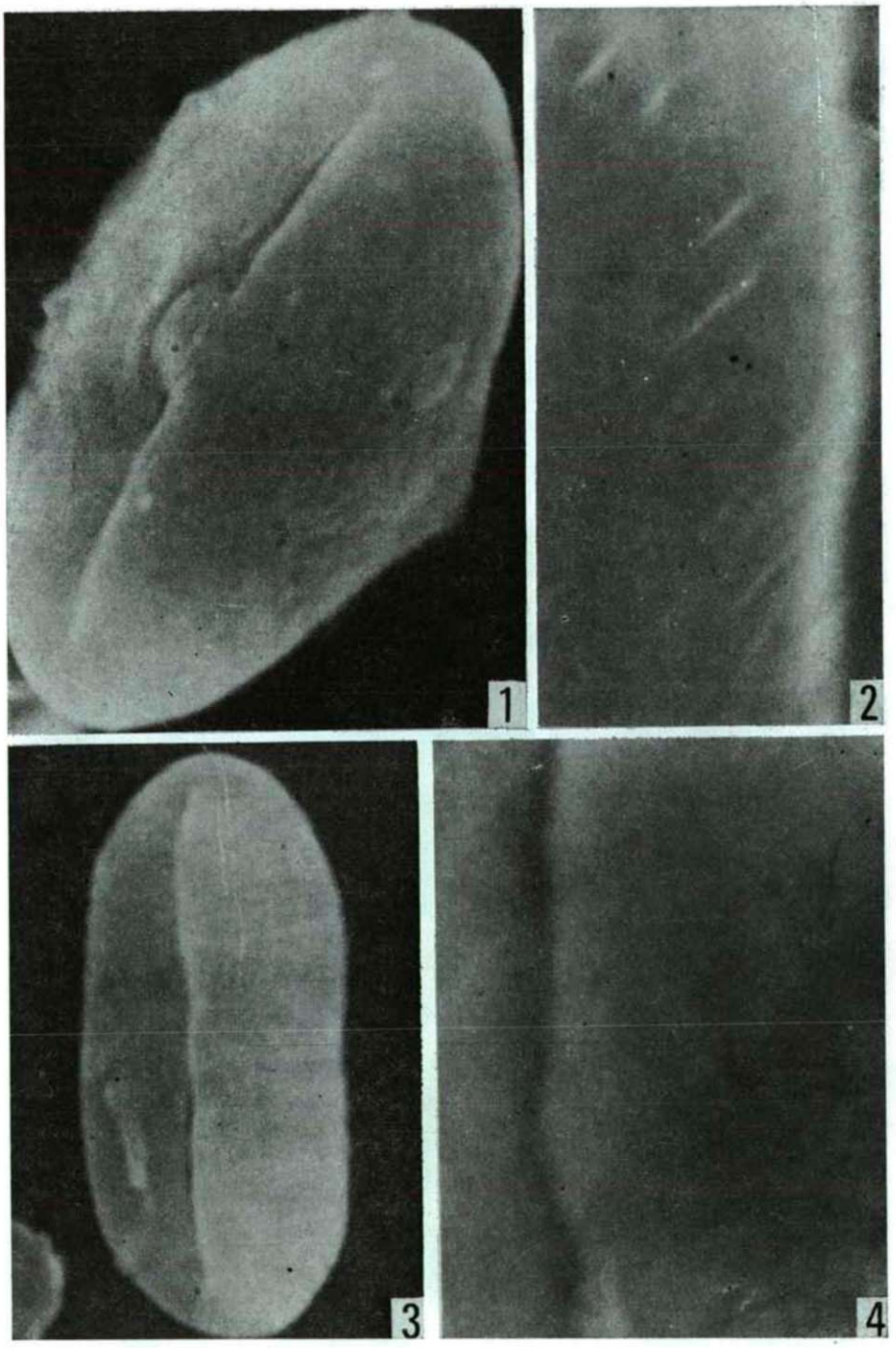

Plate VI. 1. *Castanopsis indica* DC., x5000.
2. *Castanopsis indica* DC., x10 000.
3. *Castanopsis argyrophylla* KING, x5000.
4. *Castanopsis argyrophylla* KING, x10 000.

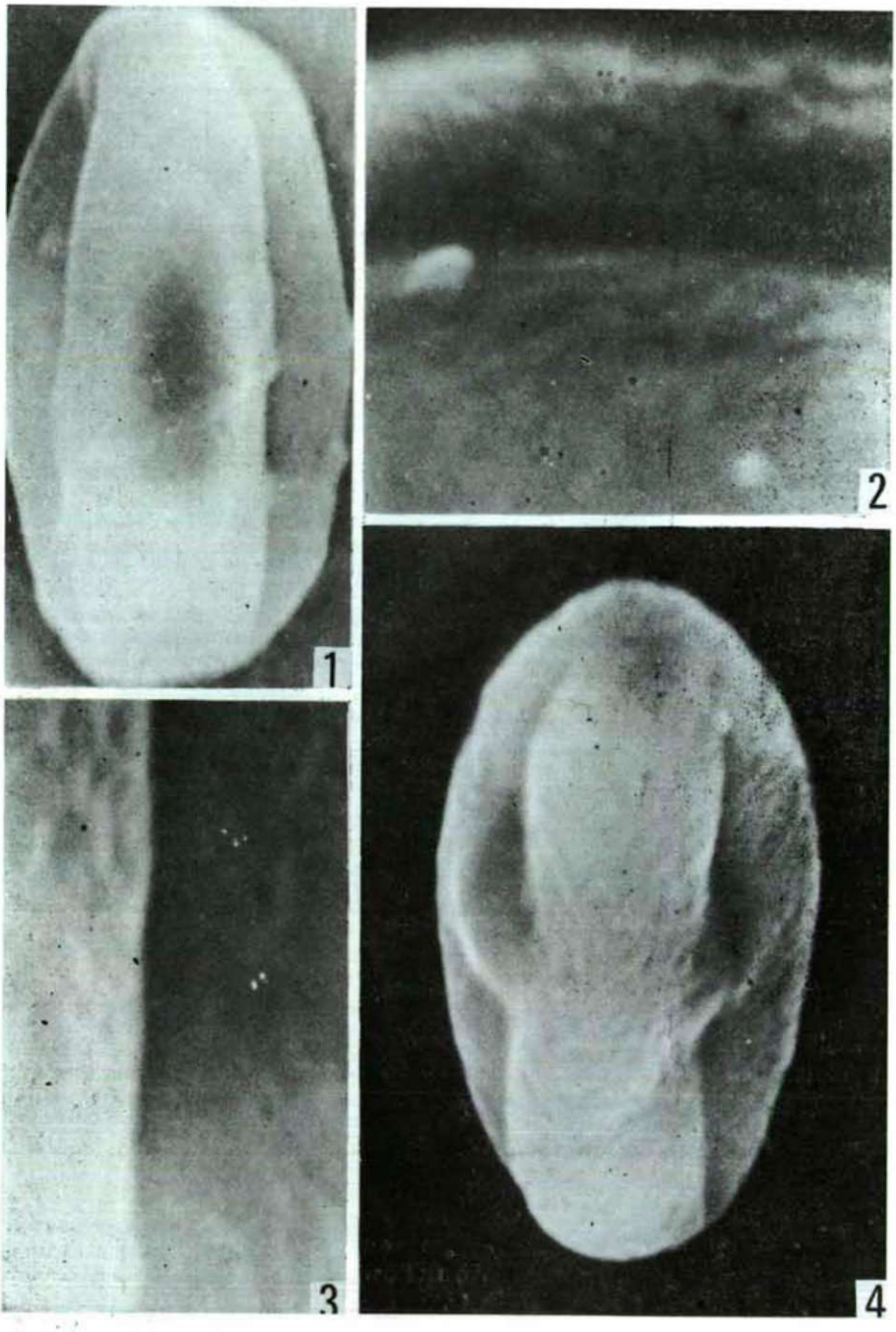

Plate VII. 1. *Pasania calathiformis* (SKAN.) H. ET C., x5000.
2. *Pasania calathiformis* (SKAN.) H. ET C., x10 000.
3. *Chrysolepis chrysophylla* (A. DC.) HJELMQVIST, x10 000.
4. *Chrysolepis chrysophylla* (A. DC.) HJELMQVIST, x5000.

9. *Chrysolepis chrysophylla* A. DC. (Plate VII, Figs. 3, 4)

The ornamentation is similar to the previous species, there is only one difference, the striate ornamentation is more frequent at this species.

Discussion

From pollen morphological point of view, the TEM structure of the caverns around the furrows is important, which is essentially in consequence of the refraction of light of the cavity (or cavities) between the ect- and endexine, or inside the endexine. This result is a partial ultrastructural interpretation of the classical lamellar ectexine stratification concept.

It is the submicroscopic striate surface, and the columellar infratectal layer which have taxonomic importance, and well separate the *Castaneoideae* pollen grains from the other *Amentiflorae* (cf. TAKEOKA 1965, DUPONT and DUPONT 1972, UENO 1975).

In respect to the evolution of the exine ultrastructure the granular endexine is worth mentioning, which is more developed, than the lamellar. But the occasional occur rence of the lamellae in the germinal area is interesting. For the importance of the lamellae the following papers are worth mentioning: CHANDA and ROWLEY (1967), ROWLEY and DUNBAR (1967), ROWLEY and SOUTHWORTH (1967), and M. VAN CAMPO and LUGARDON (1973). Endexine data about fossil *Castaneoideae* pollen grains are know from the Eocene, it may be concluded that there is no essential difference between recent and fossil endexine fine structure.

As a general conclusion it may be emphasized, that the submicroscopic characteristic features of the exine of the brevaxonate and longaxonate *Amentiflorae* pollen grains alternate with the symmetry of the polar axis. The occurrence of the endexine, and the ultrastructure of the infratectum of the different groups have different evolutionary or taxonomic significance.

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Address of the authors:
DR. M. KEDVES
Department of Botany A.J.
Univesity H-6701 Szeged,
P.O. Box 657, Hungary
DR. Á. PÁRDUTZ
Institute of Biophysics,
Biological Research Center
of the Hungarian Academy of
Science H-6701 Szeged, Hungary

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SPORES OF HUNGARIAN MIDDLE CRETACEOUS AND ITS BOTANICAL RELATIONSHIP

M. JUHÁSZ

Department of Botany, Attila József University, Szeged
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Abstract

In this paper the dispersed spores obtained from the Middle Cretaceous sediments of Transdanubia are assembled in a botanical system. The highest number of species was found in the order *Schizaeales* and in the families *Gleicheniaceae* and *Lycopodiaceae*. Fewer species represent the families *Matoniaceae, Osmundaceae* and *Selaginellaceae*. Only a few bryophyte spores were observed — mostly *Hepaticopsida*.

The cosmopolitan character of many of the species demonstrates the world-wide uniform distribution of the Early Cretaceous pteridophyte flora.
Key words: Palynology, Middle Cretaceous, Transdanubian sediments, spore taxonomy, botanical relationship.

Introduction

In the last decade several papers have been published on the botanical affinity of dispersed spores of the Cretaceous. As a basis of comparison fossil in situ sporomorphs as well as spores of recent species were used. In this way with the aid of palynological data the reconstruction of the Middle Cretaceous flora was possible.

Botanical affinity, however, can be mostly established only to the family level, except those palynological works which are based on a natural system and consider possible an identification to the generic level using analogies with the spores of recent species.

Only the discovery of additional megafossilia (and of the spores and pollen they contain in situ) can be considered as a real progress in the investigation of the 100—200 million years old flora.

At present the number of „incertae sedis" spores and pollen grains is very high and the proper botanical place of the most ancient angiospermous pollen grains is nearly totally obscure.

In this paper the sporomorphs of the Hungarian Middle Cretaceous sediments are placed in a botanical system.

In Hungary the Cretaceous sediments are found in the Central Transdanubian Mountains and in the southern parts of Transdanubia. Geological monographs on there were published by FÜLÖP (1957, 1964, 1966, 1975), synthetic evaluations of the lithological formations were given by CSÁSZÁR (1976, 1978) and CSÁSZÁR and HAAS (1979).

Results

In the following a taxonomic arrangement of the microspores and miospores of the „Sporophyta" only will be attempted.

Phylum: **BRYOPHYTA**

1. Classis: **Hepaticopsida**

Genus: Triporoletes (Mtchedlishvili 1960) Playford 1971

Triporoletes radiatus (Dett, 1963) Playford 1971.
Triporoletes reticulatus (Pocock 1962) Playford 1971.
Triporoletes simplex (Cookson et Dettmann 1958) Playford 1971.

Genus: Aequitriradites (Delc. et Spr. 1955) Cookson et Dettmann 1961.

Aequitriradites spinulosus (Cookson et Dettmann 1958) Cookson et Dettmann 1961.
Aequitriradites reticulatus Kotova 1968.

Genus: Couperisporites Pocock 1962.

Couperisporites clavatoides (Deák 1964) Juhász 1980.

Genus: Coptospora Dettmann 1963.

Coptospora cf. williamsii.
Coptospora paradoxa Dettmann 1963.

2. Classis: **Anthoceropsida**

Genus: Foraminisporis W. Kr. 1959.

Foraminisporis dailyi (Cookson et Dettmann 1958) Dettmann 1963.
Foraminisporis asymmetricus (Cookson et Dettmann 1958) Dettmann 1963.

Genus: Phaeocerosporites Nagy 1968.

Phaeocerosporites purus (Deák 1964) Juhász 1980.

3. Classis: **Bryopsida**
Familia: **Sphagnaceae**

Genus: Stereisporites Th. et Pf. 1953.

Stereisporites psilatus (Ross 1949) Pf. 1953.
Stereisporites antiquasporites (Wilson et Webster 1946) Dettmann 1963.
Stereisporites aptiensis (Deák 1964) Juhász 1980.
Stereisporites grossus Takahashi 1964.
Stereisporites europeum (Bolch. 1953) Čorna 1972.

Genus: Cingutriletes (Pierce 1961) Dettmann 1963.

Cingutriletes clavus (Balme 1957) Dettmann 1963.
Cingutriletes levispeciosus (Pf. 1953) Juhász 1980.

Subclassis: **Bryidae**

Genus: Staplinisporites Pocock 1962.

Staplinisporites caminus (Balme 1957) Pocock 1962.

Genus: Coronatispora Dettmann 1963.

Coronatispora valdensis (Couper 1958) Dettmann 1963.

The occurrence of bryophyte spores in the Hungarian Middle Cretaceous can be summarized as follows (Juhász, 1980):

these spores have an inferior role in the different sporomorph-assotiations. First of all Stereisporites, Staplinisporites and Aequitriradites occur — very sporadically and in few exemplars — in the Neocomian sediments. Most of liverwort and hornwort spores are characteristic species of the Albian rocks. They occur as locally concentrated in some boreholes as indicators of coastal swamp vegetation.

Phylum: **PTERIDOPHYTA**

1. Classis: **Lycopsida**

1. Ordo: **Lycopodiales**

Genus: Retitriletes (PIERCE 1961) D. K. M. S. 1963.
Retitriletes tenuis (BALME 1957) JUHÁSZ 1975.
Retitriletes austroclavatidites (COOKSON 1953) D. K. M. S. 1963.
Retitriletes clavatoides (COUPER 1958) D. K. M. S. 1963.
Retitriletes glebulentus (KEMP 1970) JUHÁSZ 1975.
Retitriletes dentimuratus (BRENNER 1963) JUHÁSZ 1975.

Genus: Vadaszisporites (DEÁK et COMBAZ 1967) JUHÁSZ 1975.
Vadaszisporites urkuticus (DEÁK 1964) DEÁK et COMBAZ 1967.
Vadaszisporites pseudofoveolatus (DEÁK 1964) DEÁK et COMBAZ 1967.
Vadaszisporites uniformis (SINGH 1964) JUHÁSZ 1975.
Vadaszisporites gregussi JUHÁSZ 1975.
Vadaszisporites minutireticulatus JUHÁSZ 1975.
Vadaszisporites sacali DEÁK et COMBAZ 1967.
Genus: Foveosporites BALME 1957.
Foveosporites canalis BALME 1957.
Foveosporites subtriangularis (BRENNER 1963) SCHULZ 1966.
Genus: Sestrosporites DETTMANN 1963.
Sestrosporites pseudoalveolatus (COUPER 1958) DETTMANN 1963.
Genus: Camarozonosporites (PANT ex R. POT. 1956) KLAUS 1960.
Camarozonosporites cerniidites (ROSS 1949) W. KR. 1959.
Camarozonosporites insignis NORRIS 1967.
Camarozonosporites concinnus S. K. SRIVASTAVA 1972.
Camarozonosporites Hammenii VAN AMEROM 1965

Of the Lycopodiaceae spores representants of the foveolate Foveosporites, Sestrosporites and the slightly reticulate Retitriletes are characteristic species mostly of the Neocomian sediments. Vadaszisporites and Camarozonosporites species are frequent in Albian (JUHÁSZ, 1975).

2. Ordo: **Selaginellales**

Genus: Echinatisporis W. KR. 1959.
Echinatisporis varispinosus (POCOCK 1962) S. K. SRIVASTAVA 1975.
Echinatisporis levidensis (Balme 1957) S. K. SRIVASTAVA 1972.
Genus: Cepulina MALJAVKINA 1949 ex SCHULZ 1967.
Cepulina truncata (COOKSON 1953) SCHULZ 1967.
Genus: Ceratosporites COOKSON et DETTMANN 1958.
Ceratosporites equalis COOKSON et DETTMANN 1958.
Ceratosporites rarus DÖRING 1965.
Genus: Heliosporites (SCHULZ 1962) S. K. SRIVASTAVA 1972.
Heliosporites kemensis (CHLONOVA 1960) S. K. SRIVASTAVA 1972.
Genus: Densoisporites WEYLAND et KRIEGER 1953.
Densoisporites microrugulatus BRENNER 1963.
Densoisporites velatus WEYLAND et KRIEGER 1953.

These species occur sporadically in Hungarian Middle Cretaceous; stratigraphically they are insignificant.

2. Classis: **Pteropsida**

1. Subclassis: **Osmundidae**

Ordo: **Osmundales**

Genus: Osmundacidites COUPER 1953.
Osmundacidites wellmanii COUPER 1953.
Osmundacidites densiornamentata (KLIMKO 1961) JUHÁSZ 1979.
Genus: Baculatisporites TH. et PF. 1953.
Baculatisporites comaumensis (COOKSON 1953) R. POT. 1956.
Baculatisporites kolpachevensis (KLIMKO 1961) JUHÁSZ 1979.
Baculatisporites brevibaculatus (DÖRING 1965) JUHÁSZ 1979.
Genus: Conbaculatisporites KLAUS 1960.
Conbaculatisporites cretaceus DEÁK 1964.
Genus: Todisporites COUPER 1958.
Todisporites major COUPER 1958.
Todisporites minor COUPER 1958.

Among the spores of this fern family, Todisporites, Osmundacidites, Baculatisporites species are frequent also in Jurassic. The *Conbaculatisporites cretaceus* DEÁK is characteristic in Middle Albian of Hungary.

2. Subclassis: **Polypodiide**

1. Ordo: **Schizaeales**

1. Familia: **Klukiaceae**

Genus: Ischyosporites BALME 1957.
Ischyosporites estherae DEÁK 1964.
Ischyosporites baconicus JUHÁSZ 1979b
Genus: Klukisporites COUPER 1958.
Klukisporites scaberis (COOKSON et DETTMANN 1958) DETTMANN 1963.
Klukisporites lacunus FILATOFF 1975.
Genus: Foveasporis (W. KR. 1959b) JUHÁSZ 1979b
Foveasporis agathoecus (R. POT. 1934) W. KR. 1959b
Foveasporis budejovicensis (PACLTOVÁ 1961) JUHÁSZ 1979b
Genus: Fueloepisporites JUHÁSZ 1979b
Fueloepisporites hungaricus JUHÁSZ 1979b
Fueloepisporites crassus JUHÁSZ 1979b
Fueloepisporites vokanyensis JUHÁSZ 1979b
Fueloepisporites foveasolidus (W. KR. 1967) JUHÁSZ 1979b
Fueloepisporites asolidus (W. KR. 1959b) JUHÁSZ 1979b

Detailed nomenclatural and stratigraphical elaboration of *Klukiaceae* spores is published in an earlier paper (JUHÁSZ, 1979b). Klukisporites contains some transitional species from Jurassic, most of Fueloepisporites species lived in the Neocomian; the two Ischyosporites species are common in Albian, Foveasporis is frequent in Cenomanian and Upper Cretaceous sediments.

2. Familia: **Lygodiaceae**

Genus: Concavissimisporites (DELC. et SPR. 1955) DELCOURT, DETTMANN et HUGHES 1963.
Concavissimisporites gibberulus (BOLCH. 1956) BOLCH. 1968.
Concavissimisporites verrucosus (DELC. et SPR. 1955) DELCOURT, DETTMANN et HUGHES 1963.
Concavissimisporties variverrucatus (COUPER 1958) BRENNER 1963
Concavissimisporites reticulatus (MALJAVKINA 1949) JUHÁSZ 1979.

Genus: Impardecispora VENKATACHALA, KAR et RAZA 1969.
Impardecispora apiverrucata (COUPER 1958) VENKATACHALA, KAR et RAZA 1969.
Impardecispora trioreticulosa (COOKSON et DETTMANN 1958) VENKATACHALA, KAR et RAZA 1969.
Impardecispora marylandensis (BRENNER 1963) S. K. SRIVASTAVA 1975.
Impardecispora minuta (BOLCH. 1961) JUHÁSZ 1979.

Genus: Pilosisporites DELCOURT et SPRUMONT 1955.
Pilosisporites notensis COOKSON et DETTMANN 1958.

Genus: Trilites (ERDTMAN 1947, COOKSON 1947) ex COUPER 1953.
Trilites triangulus KEDVES 1964.
Trilites knaueri JUHÁSZ 1972.
Trilites harskutensis JUHÁSZ 1972.

Genus: Pereisporites (JUHÁSZ 1972) S. K. SRIVASTAVA 1975.
Pereisporites minor (JUHÁSZ 1972) S. K. SRIVASTAVA 1975.
Pereisporites kyrtomiformis (JUHÁSZ 1972) JUHÁSZ 1979.

Genus: Bikolisporites (JUHÁSZ 1972) S. K. SRIVASTAVA 1975.
Bikolisporites toratus (WEYLAND et GREIFELD 1953) S. K. SRIVASTAVA 1975.
Bikolisporites baconicus (JUHÁSZ 1972) JUHÁSZ 1977c.
Bikolisporites distalrugulatus (JUHÁSZ 1972) JUHÁSZ 1977c.
Bikolisporites transdanubicus (JUHÁSZ 1972) JUHÁSZ 1977c.

Genus: Acritosporites (OBONIZKAJA 1964) JUHÁSZ 1979b
Acritosporites sibiricus (BOLCH. 1961) OBONIZKAJA 1964.
Acritosporites kyrtomus JUHÁSZ 1979b.
Acritosporites transdanubicus JUHÁSZ 1979b.
Acritosporites triangularis (DEÁK 1964) JUHÁSZ 1979b.
Acritosporites sibiricus (BOLCH. 1961) OBONIZKAJA 1964 *forma minor* JUHÁSZ 1979b.
Acritosporites rasellus (ALEKSANDROVA 1962) JUHÁSZ 1979b.
Acritosporites excavatus (BRENNER 1963) JUHÁSZ 1979b.

The above-mentioned *Lygodiaceae* species and their nomenclature are discussed in several papers: BOLCHOVITINA (1968), VENKATACHALA, KAR and RAZA (1969), MARKOVA (1966), JUHÁSZ (1972, 1977c, 1979b), S. K. SRIVASTAVA (1975).

In the Early Neocomian first Pilosisporites and Concavissimisporites species appeared, in Barremian the spores of Trilites and Bikolisporites. In Middle Albian the Impardecispora, while in Upper Albian-Lower Cenomanian the Acritosporites are frequent.

3.—4. Familia: **Acrostichopteridaceae — Mohriaceae**

Genus: Cicatricosisporites R. POT. et GELL. 1933.
Cicatricosisporites venustus DEÁK 1964.
C. minutaestriatus (BOLCH. 1961) POCOCK 1965.
Cicatricosisporites augustus C. SINGH 1971.
C. proxiradiatus KEMP 1970.
C. minor (BOLCH. 1961) POCOCK 1965.
C. mediostriatus (BOLCH. 1961) POCOCK 1965.
C. coconinoensis AGASIE 1969.
C. pacificus (BOLCH. 1961) JUHÁSZ 1977c.
C. potomacensis BRENNER 1963.
C. baconicus DEÁK 1964.
C. pseudotripartitus (BOLCH. 1961) DETTMANN 1963.
C. hughesi DETTMANN 1963.

Genus: Nodosisporites DEÁK 1964.
Nodosisporites verrucosus DEÁK 1964.
Nodosisporites costatus DEÁK 1964.

5. Familia: **Anemiaceae**

Genus: Plicatella MALJAVKINA 1949.
Plicatella trichacantha MALJ. 1949.
Genus: Appendicisporites WEYLAND et KRIEGER 1953.
Appendicisporites bifurcatus C. SINGH 1964.
App. crimensis (BOLCH. 1961) POCOCK 1965.
App. pseudomacrorhyzus (BOLCH. 1961) JUHÁSZ 1979.
App. potomacensis BRENNER 1963.
App. tricornitatus WEYLAND et KRIEGER 1953.
App. tricuspidatus WEYLAND et GREIFELD 1953.
App. stylosus (THG. 1949) DEÁK 1964.
App. erdtmani POCOCK 1965.
App. dentimarginatus BRENNER 1963.
App. unicus (MARKOVA 1961) C. SINGH 1964
Appendicisporites concentricus KEMP 1970.
App. cristatus (MARKOVA 1961) JUHÁSZ 1979.
Genus: Costatoperforosporites DEÁK 1962.
Costatoperforosporites fistulosus DEÁK 1962.
Costatoperforosporites triangulatus DEÁK 1962.
Costatoperforosporites foveolatus DEÁK 1962.

REED (1947) placed into this family the fossil Protornithopteris and the recent Ornithopteris, Hemianemia and Anemia species. In the palynological literature opinions differ on the question of priority between Plicatella MALJ. and Appendicisporites WEYLAND et KRIEGER. The author chares the opinion of those who accept the validity of both genera. So the triangular, on the corners strongly thickened forms without appendages are placed in Plicatella, while those with appendages in the Appendicisporites formgenera.

6. Familia: **Schizaeaceae**

Genus: Corniculatisporites KUVAEVA 1972.
Corniculatisporites virgatus (DEÁK 1963) KUVAEVA 1972.
Corniculatisporites alekhinii (BOLCH. 1953) KUVAEVA 1972.
Corniculatisporites tudariensis KUVAEVA 1972.
Corniculatisporites magniolobatus (BOLCH. 1953) KUVAEVA 1972.
Corniculatisporites bolchovitinae KUVAEVA 1972.
Corniculatisporites auritus (SINGH 1971) JUHÁSZ 1977b.
Corniculatisporites nemanicensis (PACLTOVÁ 1961) JUHÁSZ 1977b.
Genus: Cicatricososporites (TH. et PF. 1953) W. KR. 1959.
Cicatricososporites phaseolus (DELC. et SPR. 1955) W. KR. 1959.
Genus: Verrucatosporites (TH. et PF. 1953) W. KR. 1959.
Verrucatosporites contractus (BOLCH. 1953) W. KR. 1959.
Genus: Microfoveolatosporis (W. KR. 1959) R. POT. 1966.
Microfoveolatosporis baconicus JUHÁSZ 1977b.
Microfoveolatosporis surensis JUHÁSZ 1977b.
Microfoveolatosporis gallicus (DEÁK et COMBAZ 1967) JUHÁSZ 1977b.
Microfoveolatosporis csaszari JUHÁSZ 1977b.

In the system of REED (1947) which in the case of *Schizaeales* is adopted here, the recent schizaeoid species with monolete spores (Schizaea, Microschizaea, Actinostachys) were placed into the family *Schizaeaceae* sensu stricto. The spores in these recent genera have striate, verrucate-tuberculate, foveolate-microfoveolate ornamentation. Their fossil equivalents were discovered among the spores of the Albian sediments.

2. Ordo: **Filicales**

The Middle Cretaceous fern-spores show botanical relationship with the following families: **Gleicheniaceae, Matoniaceae, Hymenophyllaceae, Dicksoniaceae -Cyatheaceae.**

1. Familia: **Gleicheniaceae**

Genus: Gleicheniidites (Ross 1949) Bolchovitina 1966.

Gleicheniidites senonicus (Ross 1949) Bolch. 1968.
Gleicheniidites umbonatus Bolch. 1953) Bolch. 1968.
Gleicheniidites radiatus (Bolch. 1953) Bolch. 1968
Gleicheniidites rasilis (Bolch. 1953) Bolch. 1968.
Gleicheniidites compositus (Bolch. 1953) Deák 1964.
Gleicheniidites laetus (Bolch. 1953) Bolch. 1968.
Gleicheniidites carinatus (Bolch. 1953) Bolch. 1968.
Gleicheniidites saparicus Juhász 1977a.

Genus: Plicifera Bolchovitina 1966.

Plicifera decora (Chlonova 1960) Bolch. 1968.
Plicifera delicata (Bolch. 1953) Bolch. 1966.

Genus: Ornamentifera Bolchovitina 1966.

Ornamentifera tuberculata (Grigorjeva 1961) Bolch. 1968.
Ornamentifera granulata (Grigorjeva 1961) Bolch. 1968.
Ornamentifera peregrina (Bolch. 1953) Bolch. 1968.

Genus: Clavifera Bolchovitina 1966.

Clavifera nigra (Bolch. 1953) Juhász 1977a.
Clavifera triplex (Bolch. 1953) Bolch. 1966.
Clavifera rudis Bolch. 1968.
Clavifera tuberosa Bolch. 1968.

The systematics of the fossil trilete, tricrassate *Gleicheniaceae* was first discussed by Krutzsch (1959) who established six subformgenera inside *Gleicheniidites*.

Bolchovitina (1966, 1968) compared the recent *Gleicheniaceae* spores with the Cretaceous „*gleicheniid*" forms and establishing four new formgenera proved the relationship, too. The author followed her system in the investigation of the Hungarian Middle Cretaceous *Gleicheniaceae* (Juhász, 1977a).

2. Familia: **Matoniaceae**

Genus: Matonisporites (Couper 1958) Dettman 1963

Matonisporites major Deák 1964.
Matonisporites simplex Deák 1964.
Matonisporites minor Deák 1964.
Matonisporites weylandi (Döring 1965) Juhász 1979a.

Genus: Phlebopterisporites Juhász 1979a.

Phlebopterisporites hungaricus Juhász 1979a.
Phlebopterisporites harskutensis Juhász 1979a.
Phlebopterisporites equiexinus (Couper 1958) Juhász 1979a.
Phlebopterisporites globosus (Kimyai 1966) Juhász 1979a.

Genus: Phanerosorisporites Juhász 1979a.

Phanerosorisporites surensis Juhász 1979a.
Phanerosorisporites pectinataeformis (Dettmann 1963) Juhász 1979a.

Genus: Trilobosporites Pant ex Pot. 1956.

Trilobosporites goczani Juhász 1979a.

The family *Matoniaceae*, recently having only 3 species, was a significant element of the Early Cretaceous flora, especially in marshy areas under humid and hot climate (JUHÁSZ 1979a).

3.—5. Familia: **Cyatheaceae-Dicksoniaceae-Hymenophyllaceae**

Genus: Cyathidites COUPER 1953.
Cyathidites australis COUPER 1953.
Cyathidites minor COUPER 1953.
Cyathidites rarus (BOLCH. 1953) DEÁK 1964.
Genus: Dictyophyllidites COUPER 1958.
Dictyophyllidites harrisii COUPER 1958.

The system and botanical relationship of the laevigate trilete spores is unsolved. Only a few forms can be located into the taxonomical system.

3. Classis: **Sphenopsida**

Genus: Calamospora SCHOPF 1944.
Calamospora mesozoica COUPER 1958.

Sporae Incertae Sedis

Genus: Deltoidospora MINER (1935) R. POT. 1956.
Deltoidospora diaphana (WILSON et WEBSTER 1946) JUHÁSZ 1979.
Deltoidospora ordinata (BRELIE 1964) JUHÁSZ 1979.
Deltoidospora juncta (KARA-MURZA 1954) C. SINGH 1964.
Genus: Undulatisporites PFLUG 1953.
Undulatisporites pannuceus (BRENNER 1963) C. SINGH 1971.
Undulatisporites undulapolus BRENNER 1963.
Undulatisporites sculpturoides PF. 1953.
Genus: Obtusisporis (W. KR. 1959) POCOCK 1970.
Obtusisporis jurienensis (BALME 1957) JUHÁSZ 1979.
Obtusisporis mesozoicus KDS et SCSICS 1964.
Genus: Varirugosisporites DÖRING 1965.
Varirugosisporites lentiformis DÖRING 1965.
Varirugosisporites proxigranulatus (BRENNER 1963) JUHÁSZ 1979.
Varirugosisporites pseudogibberulus (BOLCH. 1961) JUHÁSZ 1979.
Varirugosisporites verrucosus (DEÁK 1964) JUHÁSZ 1979.
Genus: Gemmatriletes VAN DER HAMMEN 1954.
Gemmatriletes irregularis (BRENNER 1963) JUHÁSZ 1979.
Genus: Leptolepidites (COUPER 1953) NORRIS 1969.
Leptolepidites verrucatus COUPER 1958.
Leptolepidites psarorus NORRIS 1969.
Genus: Rubinella MALJAVKINA 1953.
Rubinella major (COUPER 1958) NORRIS 1969.
Genus: Clavatisporites KDS et SCICS 1964.
Clavatisporites rotundiformis (KRASNOVA 1961) JUHÁSZ 1979.
Genus: Rotverrusporites DÖRING 1965.
Rotverrusporites brevilaesuratus (POCOCK 1962) DÖRING 1965.
Genus: Duplexisporites DEÁK 1962.
Duplexisporites generalis DEÁK 1962.
Genus: Vinculisporites DEÁK 1964.
Vinculisporites flexus DEÁK 1964.
Genus: Distaltriangulisporites C. SINGH 1971.
Distaltriangulisporites perplexus (C. SINGH 1964) C. SINGH 1971.

Genus: Asbeckiasporites VON BRELIE 1964.
Asbeckiasporites wirthii BRELIE 1964.
Genus: Antulsporites ARCHANGELSKY et GAMERRO 1966.
Antulsporites distaverrucosus (BRENNER 1963) ARCHANGELSKY et GAMERRO 1966.
Genus: Trubasporites VAVRDOVA 1964.
Trubasporites foveolatus (COUPER 1958) VAVRDOVA 1964.
Genus: Collarisporites DEÁK 1964.
Collarisporites fuscus DEÁK 1964.
Genus: Laevigatosporites (IBRAHIM 1933) R. POT. et KREMP 1954.
Laevigatosporites ovatus WILSON et WEBSTER 1946.

Conclusions

The above taxonomical list shows the presumable botanical relationship of the spores discovered and identified in the Transdanubian Middle Cretaceous sediments.

It gives a qualitative picture about the plant groups which lived on the lands bordering the assemblages. It can be established:

1) In the Middle Cretaceous the spores of ferns show higher number of species than of pollen gymnosperms and angiosperms;

2) the most variable and rich in species in the order *Schizaeales*, which dominated at this time on the whole hemisphere;

3) inside the order *Schizaeales* a sequence of evolution can be observed: the most ancient is the family *Klukiaceae*, the *Anemiaceae* culminated typically in Lower Cretaceous, the most longevous is the *Mohriaceae-Acrostichopteridaceae* (its spores are frequent in Tertiary, too) the youngest is the Schizaeaceae with monolete spores evolved in the Albian;

4) the *Gleicheniaceae* and *Matoniaceae* are very important components of the Middle Cretaceous. The family *Gleicheniaceae* occurs all-over the world, on some places (Russian Platform, Crimea) it is dominating in the Aptian and Albian. The family *Matoniaceae* is more bound to given environmental-climatic conditions and so is a locally accumulating component;

5) besides *Filicales* the representatives of *Lycopodiaceae* are significant. In the Transdanubian assemblages *Retitriletes* and *Foveosporites* in the Neocomian, *Vadaszisporites* and *Camarozonosporites* species in the Albian are frequent;

6) although laevigate spores are frequent, due to their unsolved botanical affinities no opinion can be formed about the role of families *Cyatheaceae, Dicksoniaceae, Hymenophyllaceae* and *Cheiropleuriaceae* in the Cretaceous — at present they are significant in the tropical fern-flora;

7) the *Bryophyta* spores are insignificant in the Hungarian Early Cretaceous; the spores of the *Hepaticopsida* occur in greater number;

8) among the studied *Bryophyta* and *Pteridophyta* spores relatively few are endemic, the majority of the spores occur in the North American, Asian, some of them in the Australian Early Cretaceous sediments, too. That leads us to believe that before the explosion-like radiation of the angiosperms in the Upper Cretaceous, in the Lower and Middle Cretaceous a more or less homogenous flora with many cosmopolitan species on the land;

9) from the recent *Pteridophyta* families the *Gleicheniaceae, Matoniaceae, Anemiaceae, Schizaeaceae* and even the majority of *Lycopodiaceae* live on tropical-subtropical areas, the number of species is not very high, the ecological limits are some-

times narrow. Their occurrence in the Cretaceous all over the world suggests that the representatives of these families in those times had a great ecological and they showed much higher adaptability than the recent species do.

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Address of the author:
Dr. M. Juhász
Department of Botany A. J. University
H-6701 Szeged, P. O. Box 657
Hungary

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THE EFFECT OF PRECIPITATION MAXIMUMS ON THE SPECIES SYNTHESIS OF THE AGROPHYTOCENOSES OF THE SAND RIDGE BETWEEN THE DANUBE AND TISZA

GY. BODROGKÖZY

Department of Botany, Attila József University, Szeged
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Abstract

The study was carried out in the Southern regions of the sand ridge between the Danube and Tisza of the Great Hungarian Plain in the years when the precipitation reached the maximum in June. On its effect inland waters developed for shorterlonger periods even in the sandy croplands. Due to this the original agrophytocenoses changed. During the course of evaluating the changes in their species synthesis 30 subunits were separated within 10 categories according to moisture demand. These values were compared with the data of the F and W values.

Correlation was found between certain Consolido-Eragrostion minoris and Tribulo-Eragrostion minoris associations and the range of their soil- and subtypes.

With the help of the vegetation map prepared in the area of the village Tázlár conclusions may be drawn regarding the various degrees of the danger of inland waters, which may also be useful in practice.
Key words: Hungary, sand ridge between the Danube and Tisza, agrophytocenoses, hydroecology.

Introduction

In the relations of the lowland, from the environmental-biological factors water has the most important effect on the species synthesis of the stands. This is valid both in the case of naturally occurring and agrophytocenoses. The sand areas between the Danube and Tisza proved especially suitable for studies in this concern, since these areas are of different relief and are characterized by various stands developing on the effect of inland waters following the precipitation maximums appearing in the beginning of Summer.

The question of classification according to the moisture demand of plant species has come into foreground of interest both in Hungary and abroad. The classification of the Hungarian flora according to species — taking as a base the system of ELLENBERG — was accomplished by SOÓ and published in six volumes (1964—1980), taking into consideration their temperature and nitrogen demands, too. This work was made more accurate by the T W and R scale of ZÓLYOMI and his co-workers (1966) comprising 1400 species. However, while the work of SOÓ uses the No. 5 scale system and the transitional units within this, supplemented with the O category unit, ZÓLYOMI et al. grouped the processed species into 11 categories. Here the O unit received another meaning, and the unit of the indifferent species was eliminated. The mathematical calculations in this regard were carried out by PRÉCSÉNYI.

For the further improvement of the classification of plant species according to water-demand the elaboration of the basis of such a hydroecological system became necessary, where three-three; that is a total of thirty subunits can be disintegrated within the ten categories. This, however, can only be accomplished if the H-curve of the

various species is also designed (BODROGKÖZY, 1982). With the use of these graphs their distribution according to the quota of their basal area within the different stands can also be determined.

Materials and Methods

The chosen area surveyed during the course of studying is situated in the Southern region of the sand ridges between the Danube and Tisza in the plain of Bócsa-Tázlár-Kiskunhalas (Fig 14). In this area of different relief the grass plants of the puszta covering the sand-hill systems (Festucion vaginatae) vary from hawthorn thickets to injured acacia groves. The tilling of arable land as well as grape-vine and fruit production are being carried out in the areas of more shelving relief. The meadow sand and moulding mud soil of the flat lands range from marsh meadow associations to saline plains having szoloncsák and szloncsák-szolonyec soil, respectively.

In this paper, fisrstly the questions related to the culture cenoses developing on the various soil sypes, and the classification of their species components according to moisture demand will be discusted. Furthermore, a report is given on the possibilities of applying supplementary methods related to the determination of their water demand.

The elaboration from a similar viewpoint of the sandy plain stand was carried out near Szeged, in the Nature Conservation Area of Ásotthalom (BODROGKÖZY, 1982).

During the procession of the data originating from the various culture cenoses not receiving weedicide, each species component's graph was prepared reflecting their hydroecological demand (hereinafter H). On the graph, the closer the minimum values get to each other, the higher the percentage of the culmination point is. Generally the species reaching a maximum value of 50% or more have a restricted H-amplitude. Thus, from this viewpoint these can be considered as the H-characteristic species components of the various phytocenoses, and conversely (Fig. 1).

For the purpose of comparison the F (ELLENBERG, Soó system) and W numbers (ZÓLYOMI et al. 1966) were also given in the tables of the different agrophytocenoses in case of the species belonging to the various H-categories. Their comparison is made difficult because of the varying scale-grades, nevertheless, it could be determined that despite the evaluations carried out with different methods their classification coincides. Differences appeared in the case of the species with wider H-compyling capacity (BODROGKÖZY, 1982). — The classification of 10 H-categories elaborated by the author wished to serve as a further improvement, within which 30 subunits were separated with the help of the H-curves to demonstrate the borderlines between the different categories. No. 1 indicates a transition towards a damper neighbouring category, No. 3 indicates that of a drier neighbouring category, while No. 2 gives an indication of the type (Table 1., Figs. 3, 6).

The natural features of the studied area are suitable for the congregation of inland waters developing quite frequently in places on the effect of precipitation maximums in the beginning of Summer in Hungary. Later, depending on their duration, smallerlarger qualitative or quantitative changes occur in the species composition of the various culture phytocenoses on these areas of deeper relief.

Cenosystem of the stands

During the systematization ol the processed associations and their smaller units, resp., besides the agrophytocenoses the associations referred to are listed, in an unusual manner.

CHENOPODIO-SCLERANTHEA

Secalietea

Eragrostetalia

Tribulo-Eragrostion minoris

1. *Vicio-Polygonetum arenarii* TIM. 57.
2. *Tribulo-Tragetum* SOÓ et TIM. 54.
3. *Vicio-Eragrostietum minoris* TIM. 57.
4. *Digitario-Portulacetum* (Felf. 42) TIM. et BODRK. 55.
5. *Portulaco-Chenopodietum* (n. nov.)
6. *Hibisco-Eragrostietum minoris* SOÓ et TIM. (51) 57.

Consolido-Eragrostion minoris
7. *Amarantho — Chenopodietum albi* (MORARIU 43) Soó 53

Bidentetea tripartitae
Bidentetalia
Chenopodion rubri
8. *Lythro — hissopifoliae — Gnaphalietum luteo-albi* (BODRK. 48) PIETSCH 64

PUCCINELLIO-SALICORNEA
Festuco-Puccinellietea
Puccinellietalia
Juncion gerardii
9. *Agrostio-Caricetum distantis* (RAPCS. 27) Soó 30

Results and discussion

The following culture cenoses were processed, starting from the higher relief conditions, taking into consideration their changes in succession (Fig. 13):

1. *Vicio — Polygonetum arenarii*

These are Autumn spiked cultures with loose, lime carbonate quick-ground, they are firstly the weed association of rye-sowings since other spiked species can only rarely be grown profitably in its site.

This humus-poor, chalky, loose sand-drift becomes reorganized frequently in the Summer draughty period, on the effect of which the formed organic matter becomes oxidized rapidly and so only 0.5 % can be demonstrated in the rooting zone. The washable anount of the soil physical fraction is extremely low.

Its moisture supply is only satisfactory in the aspects of Spring, early Summer and late Autumn. The precipitation maximum developing at times in June may favourably influence the qualitative and quantitative constitution of the certain cenoses, nevertheless, the species needing moisture do not find their conditions for life. From their character species, the denominating *Polygonum arenarium, Vicia villosa* and

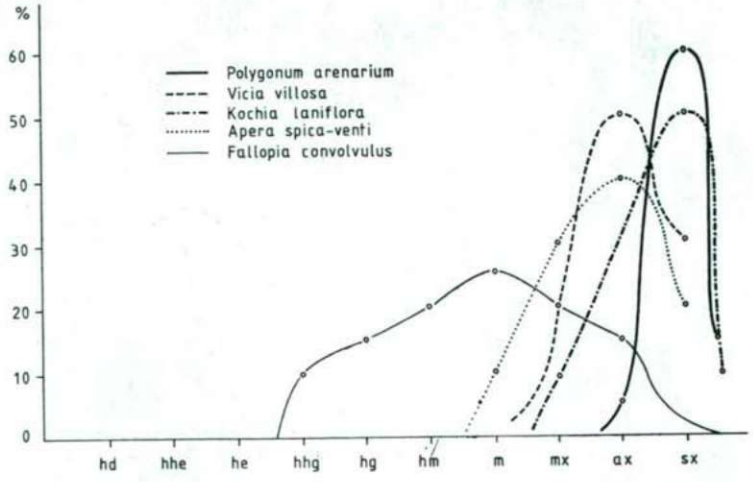


Fig. 1. H-curves of the species components of loose quick ground Autumn spiked cultures.

Kochia laniflora have H-curves where the maximum is over 50% or at least reaches this value; therefore these can be regarded as species having restricted H-amplitude. On the basis of their total covering quota the *sx 3* — species showed by far outstanding values (Fig. 3).

Earlier, in the arable, grape-vine and fruit cultures left out of the cultivation periodically, the succession of reestablishment of the plain vegetations was observable in the period without cultivation, depending on its duration. In such a way the primary sandy grassland shows a development from *Brometum tectorum* towards *Festucetum vaginatae danubiale*. Here, too, as in the other sandy plain areas of the lowland, the poineer species is the extremely compliant *Erigeron canadensis*, until it preserves its competitiveness; and its association with the *Ambrosia elatior* has been observed recently. These were later followed by the *Euphorbia seguieriana, Centaurea arenaria* ssp. *tauscheri*. From the aspect of the stubble-field the *Vicio-Polygonetum arenarii* showed a transition towards the following row crop weed association.

2. *Tribulo — Tragetum*

This is the weed association of loose, chalky quick ground row crop cultures. Since in this site most of the row crop cultures cannot always be cultivated profitably, the grape-vine cultures giving the best wine grown on sandy soil were established here. Its nutriment and water supply is partially similar to that of the afore-mentioned spiked cultures. The fact, however, that the vegetative period of these row crop weed cenoses falls to the Summer draughty period causes significant changes. Accordingly, apart from the increased insolation these species components also have to stand the almost unbearable degree of warming up of the soil surface as well as the damaging effect of this. The unfavourable site conditions can be decreased by intensive organic matter replacement and watering, resp. The H-relations are essentially more extreme than those of the spiked crops having similar site conditions. The higher H-culmina-

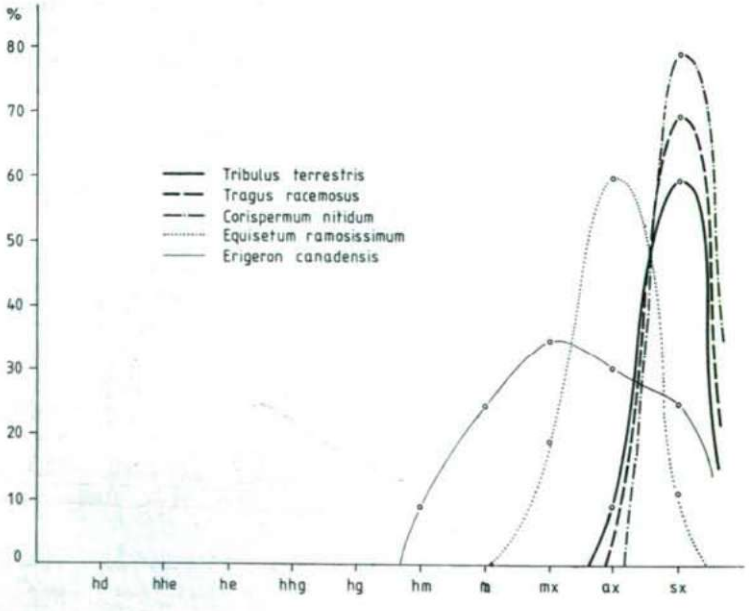


Fig. 2. H-graph of *Tribulo-Tragetum* species.

tive point of certain species can be explained by this. Thus, for example, that of the *Corispermum nitidum* may even reach the value of 80%. This is followed by the *Tragus racemosus* and the *Tribulus terrestris*. Their further data are shown on Fig. 2.

Comparing the covering quota of the *Tribulo-Tragetum* species and the spiked cultures, major difference could not be demonstrated within the H-categories. The *sx 3* is also outstanding in this case (Fig. 3). However, while the majority of the occurring

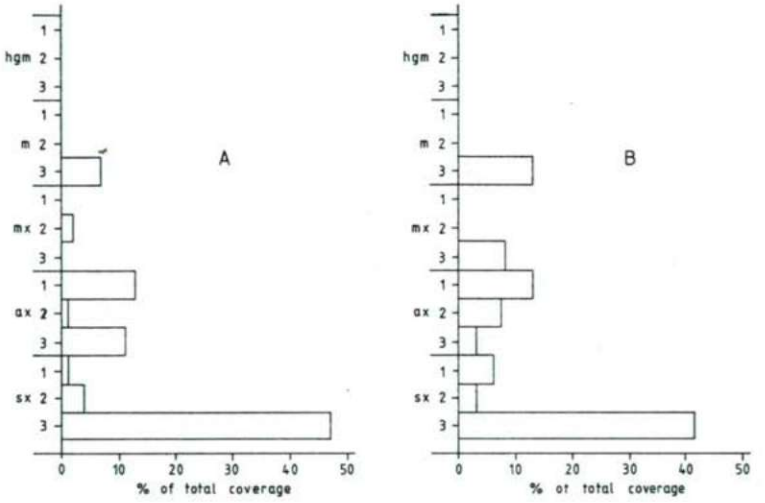


Fig. 3. Comparative graphs of the total covering values of the *Vicio-Polygonetum arenarii* (A) and *Tribulo-Tragetum* species, (B) within H-category subunits.

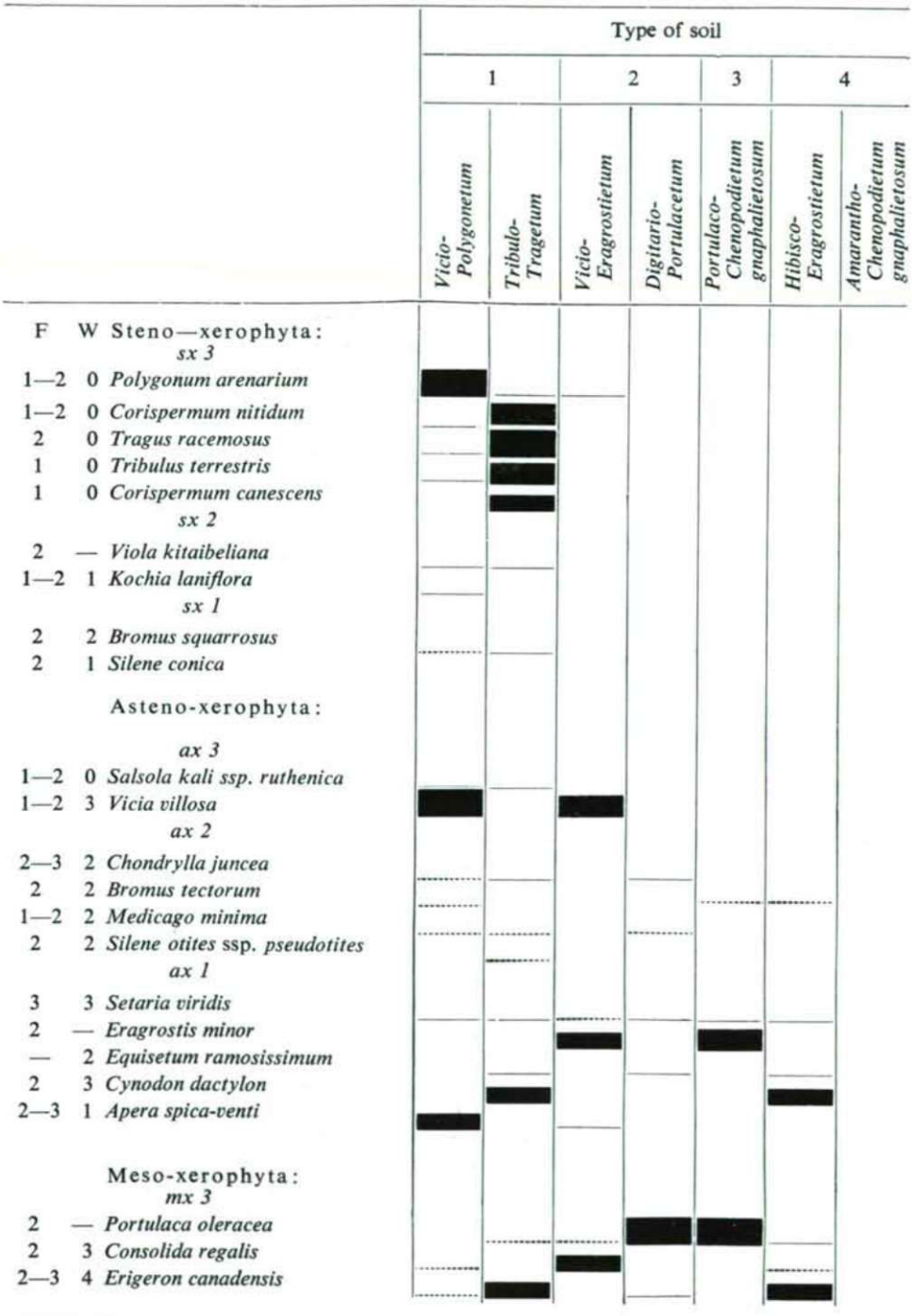
weed species belongs to one of the subunits of the *ax* and *sx* H-categories, the significant covering quota is striking in the case of certain *m 3* species having wide H-ecological compliance. Examples of this are the *Chenopodium album* and the *Fallopia convolvulus* (Table 1, Fig. 3). The appearance of *Portulaca oleracea* subass is of transitional character (Table 1).

3. *Vicio-Eragrostietum minoris*

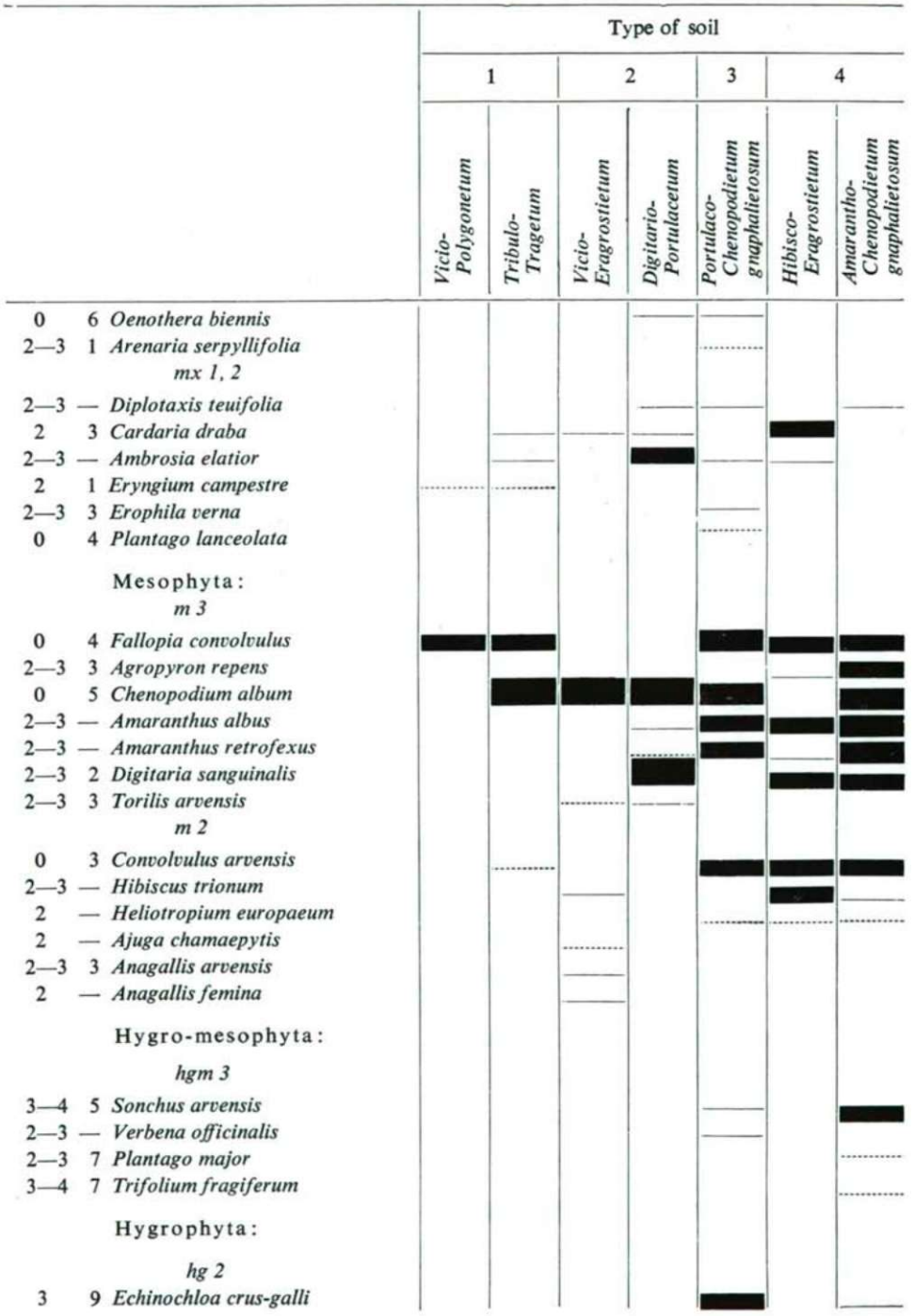
The spiked cultures with soil of harder lime carbonate and humous quick ground belong to this association. Their soil profile is homogeneous. Since the Summer reestablishment of the soil does not take place, there is a possibility for soil development. This is mainly manifested in the accumulation of the organic matter content. Its amount, however, does not reach 1% even near the surface. Its water-binding capacity is favourably influenced by the increasing amount of the washable fraction. The low soil-stagnant water content means an advantage for its stand.

Analysis of its species composition showed that the dominating components have essentially wider H-compliance than the previous two associations. According to this, the culminative point of their H-curve does not reach the value of 50%. The only exception was the *Vicia villosa*. The widest H-amplitude was that of the *Consolida regalis: ax3, mx3, 2* (Fig. 4).

Table 1. Distribution according to water demand of the species components of field crop associations in the study area



F	W	Steno—xerophyta:
		sx 3
1—2	0	*Polygonum arenarium*
1—2	0	*Corispermum nitidum*
2	0	*Tragus racemosus*
1	0	*Tribulus terrestris*
1	0	*Corispermum canescens*
		sx 2
2	—	*Viola kitaibeliana*
1—2	1	*Kochia laniflora*
		sx 1
2	2	*Bromus squarrosus*
2	1	*Silene conica*
		Asteno-xerophyta:
		ax 3
1—2	0	*Salsola kali ssp. ruthenica*
1—2	3	*Vicia villosa*
		ax 2
2—3	2	*Chondrylla juncea*
2	2	*Bromus tectorum*
1—2	2	*Medicago minima*
2	2	*Silene otites* ssp. *pseudotites*
		ax 1
3	3	*Setaria viridis*
2	—	*Eragrostis minor*
—	2	*Equisetum ramosissimum*
2	3	*Cynodon dactylon*
2—3	1	*Apera spica-venti*
		Meso-xerophyta:
		mx 3
2	—	*Portulaca oleracea*
2	3	*Consolida regalis*
2—3	4	*Erigeron canadensis*


Type of soil
1
2
3
4
Vicio-Polygonetum
Tribulo-Tragetum
Vicio-Eragrostietum
Digitario-Portulacetum
Portulaco-Chenopodietum gnaphalietosum
Hibisco-Eragrostietum
Amarantho-Chenopodietum gnaphalietosum
0 6 Oenothera biennis
2—3 1 Arenaria serpyllifolia
mx 1, 2
2—3 — Diplotaxis teuifolia
2 3 Cardaria draba
2—3 — Ambrosia elatior
2 1 Eryngium campestre
2—3 3 Erophila verna
0 4 Plantago lanceolata
Mesophyta:
m 3
0 4 Fallopia convolvulus
2—3 3 Agropyron repens
0 5 Chenopodium album
2—3 — Amaranthus albus
2—3 — Amaranthus retrofexus
2—3 2 Digitaria sanguinalis
2—3 3 Torilis arvensis
m 2
0 3 Convolvulus arvensis
2—3 — Hibiscus trionum
2 — Heliotropium europaeum
2 — Ajuga chamaepytis
2—3 3 Anagallis arvensis
2 — Anagallis femina
Hygro-mesophyta:
hgm 3
3—4 5 Sonchus arvensis
2—3 — Verbena officinalis
2—3 7 Plantago major
3—4 7 Trifolium fragiferum
Hygrophyta:
hg 2
3 9 Echinochloa crus-galli

			Type of soil						
			1		2		3	4	
			Vicio-Polygonetum	*Tribulo-Tragetum*	*Vicio-Eragrostietum*	*Digitario-Portulacetum*	*Portulaco-Chenopodietum gnaphalietosum*	*Hibisco-Eragrostietum*	*Amarantho-Chenopodietum gnaphalietosum*
4—5	8	*Ranunculus repens*							
3—4	6	*Potentilla reptans*							
		hg 1							
2—3	7	*Linum catharticum*							
4	9	*Chlorocyperus glomeratus*							
4	6	*Tetragonolobus maritimus*							
4	8	*Mentha pulegium*							
		Helo-hygrophyta:							
		hhg 3							
3—4	—	*Gnaphalium luteo-album*							
3	8	*Agrostis stolonifera*							
4	7	*Blackstonia acuminata*							
3	7	*Achillea asplenifolia*							
4	8	*Pycreus flavescens*							
3—4	9	*Bidens tripartita*							
4—5	9	*Lycopus europaeus*							
		hhg 2							
4	10	*Cyperus fuscus*							
4—5	7	*Potentilla anserina*							
3	8	*Centaurium vulgare* ssp. *uliginosum*							
		hhg 1							
4	10	*Juncus articulatus*							
4—5	10	*Eleocharis palustris*							
		Helophyta:							
		he 3, 2							
4—5	9	*Mentha aquatica*							
4	9	*Teucrium scordium*							

The species distribution according to the various H-categories and their situation within the association, resp. can be evaluated on the basis of their covering quota. According to this, here the transitional *ax 1* as well as the species belonging to the *mx*

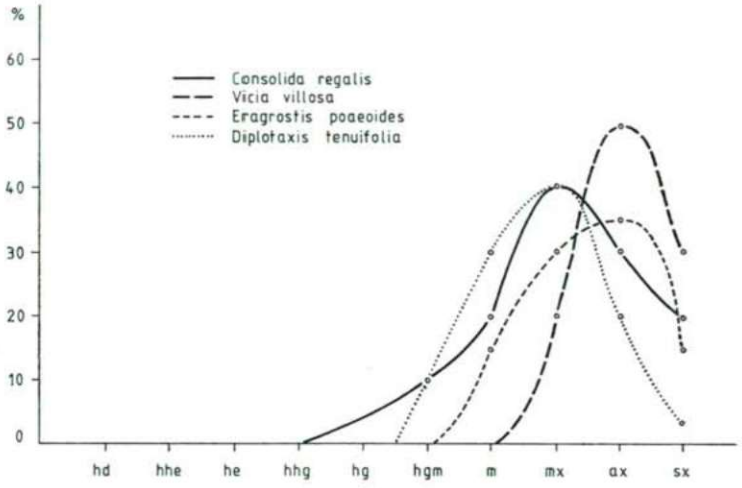


Fig. 4. H-curves of hard quick ground spiked species.

cultures (although giving wine of poorer quality) and orchards — firstly peacheries and *m* categories play the leading role, and not the *sx*.

From the *mx* and *m* categories the transitional-type *m 3 Agropyron repens* became prevalent. Further details can be seen on Table 1 and Fig. 6.

4. *Digitario-Portulacetum*

This association is made up of hard lime carbonate quick ground Autumn spiked areas, mainly row crop weed cenoses developed in the areas of row crops. It gives large crops and is also the characteristic weed cenosis of the regularly cultivated grape-vine and apple cultures (BODROGKÖZY, 1958, 1959).

Regarding the affiliation within the H-categories of the various species components it could be determined that the dominating species are those having wider H-ecological compliance. Accordingly, the maximum value of their H-curve did not rise above 40%. By now the amount of the mesophyton representatives is significant (Fig. 5).

During the course of analysing their total covering quota it was demonstrable that compared to the previous weed ceoan cenosis the percentage value of the *m 2* members decreased to the advantage of the *m 3* representatives. This was firstly due to the values of the *Digitaria sanguinalis* and the *Agropyron repens,* reaching high covering values during the late Summer.

Within the *mx*-category, an outstanding value was shown by the *Portulaca oleracea* beloing to *mx 3,* also becoming prevalent by the end of Summer (Fig. 6). Besides this, the also continuously regerminating *Ambrosia elatior* of the *m 2* group may have a role, but only if certain weedings are left out.

In the case when due to some kind of cause the arable-, grape-vine- or orchard cultures are not cultivated — which was mainly observable in the previous period —

transitionally the dense stands of *Erigeron canadensis* and *Ambrosia elatior* appear for the period of a few years, suppressing the rest of the weed species. Nevertheless, in the Spring aspect the yellow carpet-bed of *Senecio vernalis* covers these areas.

In the study area the hard quick grand spiked and row crop cultures can still be found between such high relief conditions that they do not always fall under the effect of the overflows in the years when inland waters occur. Therefore hygro- and helo-hygrophyton representatives do not appear among their species components.

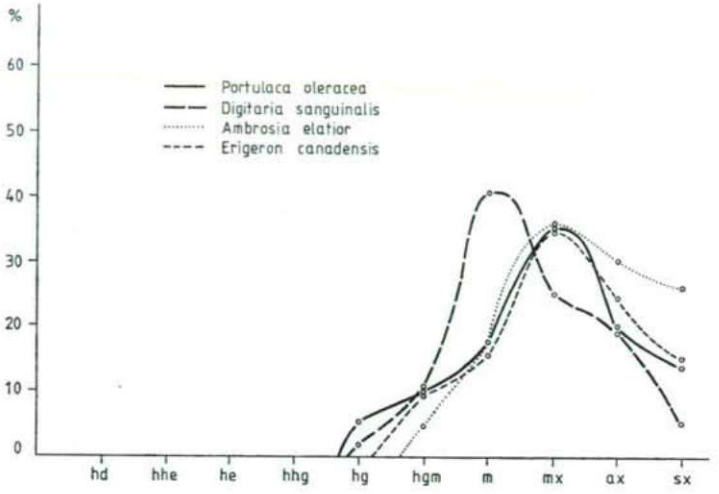


Fig. 5. Graph of H-characteristic species of the *Digitario-Portulacetum*.

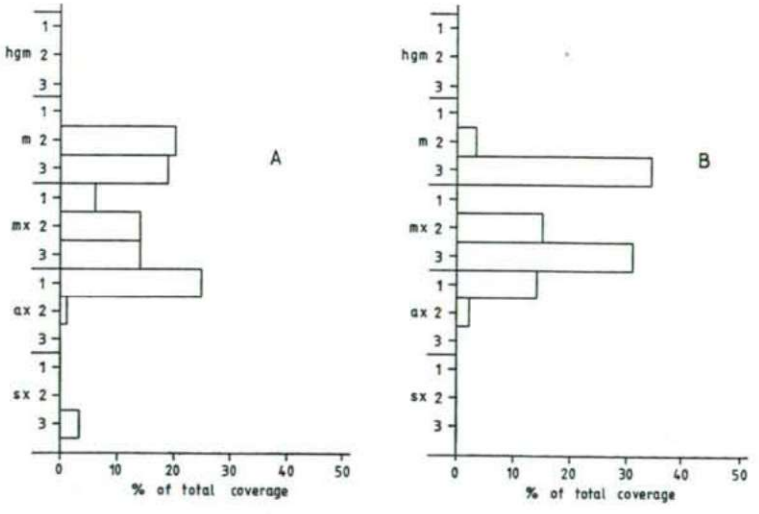


Fig. 6. Comparative graphs of the total covering quota of the *Vicio-Eragrostietum minoris* (A) and *Digitario-Portulacetum* species (B).

5. *Portulaco-Chemopodietum albi gnaphalietosum*

These are firstly the culture cenoses of the humous sandy soil row crop cultures; potato, maize, sunflower, but also of paprika. The organic matter content of their soil is between 1.5—2%, depending on the nutriment supply. In regard of its granule composition the washable fraction became the double compared to the previous one. As the consequence of the changed site conditions species of higher standard also enter into their cenoses without a change in the dominating role of *Portulaca oleracea.* Such species are the *Chenopodium album, Amaranthus albus, A. retroflexus, Fallopia convolvulus,* belonging to the *m 3* group (Fig. 7).

In respect of their covering quota the species of *mx 3* and *m 3* reached outstanding values (Fig. 9).

Following the development of precipitation maximum in the beginning of the Summer the gathered inland waters in this zone affect the stands of *Portulaco-Chenopodietum* only for a short time. Neither the culture vegetation, nor the weed species suffer damage on its effect. However, in the second half of the Summer certain helo-phyton representatives, mainly the *Gnaphalium luteo-album, Pycreus flavescens* and possibly the *Cyperus fuscus* may have a role individually or with low covering quota in the lower relief zones of these humous sandy row crop cultures. Despite the fact the culminative points of the H-curves of these mud plant species vary between the values of 50—70% in the *hhg* category, they also find conditions for life between the *hgm* conditions. This is particularly valid in the case of *Gnaphalium luteo-album* (Fig. 7).

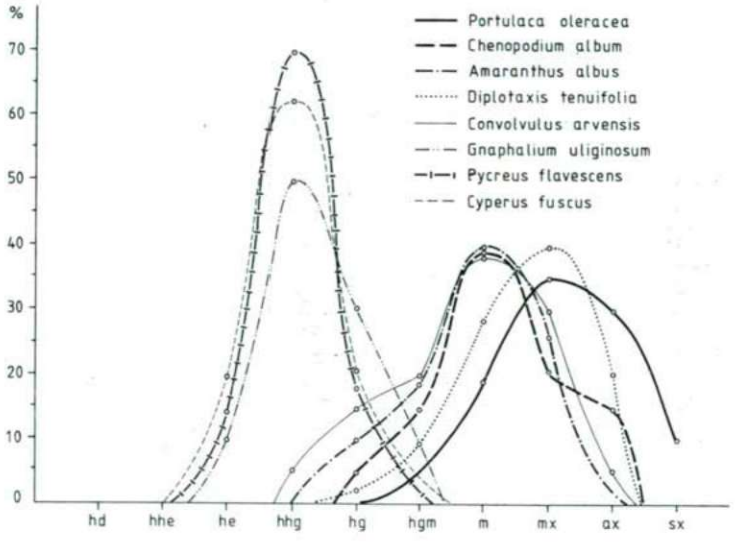


Fig. 7. Comparative H-curves of the *Portulaco-Chenopodietum albi gnaphalietosum* species.

6. *Hibisco — Eragrostietum minoris*

In the study area, and also in other regions of the country, this is the weed association of the chernozem-type sand as well as the chernozem-soiled Autumm spiked cultures. The stands are formed by frequent species germinating in Autumn and gi-

ving crops during the course of the Summer. These species also have wide H-compliance. The maximum points of their H-curve are mostly under the value of 40%. They reach the total covering maximum in the aspect of the stubble-field, when a transition occurs towards the row crop weed associations developing under similar site conditions (Fig. 9). At times of inland waters they may be covered by these waters for shorter-longer period in the areas of deeper relief, mainly in the aspect of the stubble-field. If this phenomenon is only of short duration the subassociation described in the case of *Portulaco-Chenopodietum albi* may develop.

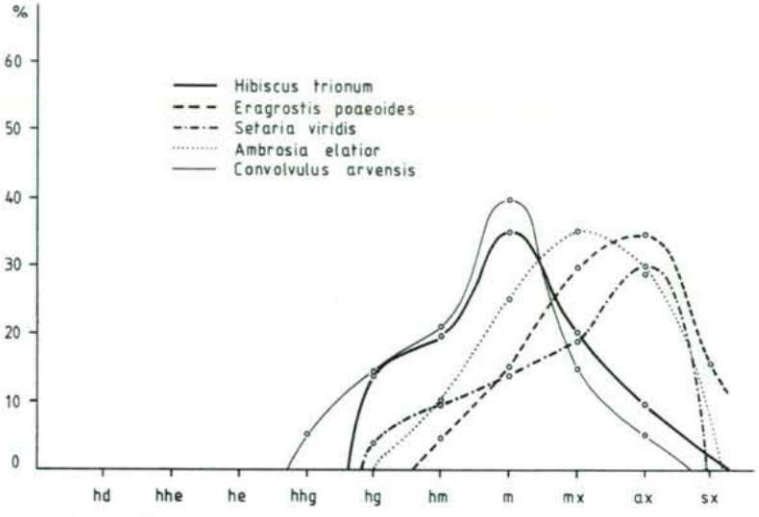


Fig. 8. H-curves of the *Hibisco-Eragrostietum minoris* species.

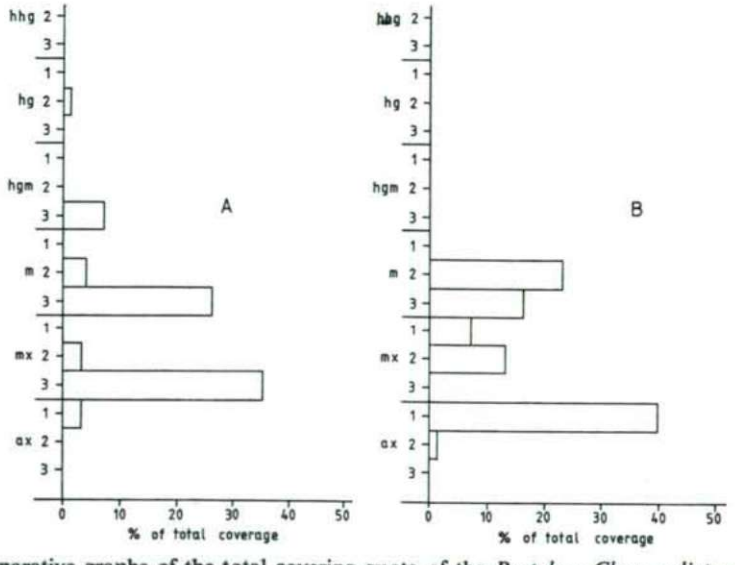


Fig. 9. Comparative graphs of the total covering quota of the *Portulaco-Chenopodietum gnaphalietosum* (A) and *Hibisco-Eragrostietum* species, (B), within the H-categories.

7. *Amarantho-Chenopodietum albi gnaphalietosum*

In the study area it is of subordinate position; just like in the case of it's spiked partners it regularly occurs in the harder meadow-like sand of chernozem character and in the chernozem soils, resp. Accordingly it forms the culture cenoses of potato, maize and cattle-turnip. It is one of the most wide-spread arable soil and garden weed cenoses all over the country.

Regarding its situation it forms the deepest relief zone here. Therefore this area is exposed to the effects of inland waters in a greater extent. From hydroecological point of view, contrary to the species components of the *Hibisco-Eragrostietum minoris,* here the H-characterized members belong to the *m*-category. Accordingly the *Amaranthus albus, A. retroflexus, Agropyron repens, Chenopodium album,* etc. can be regarded as *m 3* representatives. Their total covering quota is also expressedly high (Fig. 11).

In periods of inland waters the largest amount of helohygrophyta was detectable in this area, but helophytons also entered the cenoses individually, as the *Mentha aquatica, Teucrium scordium.* From these the *hhg 2 Cyperus fuscus* and *Potentilla anserina* appeared as a subassociation besides the denominating *Gnaphalium luteo-albun* (Fig. 10).

8. *Lythro hyssopifoliae — Gnaphalietum luteo-albi gnaphalietosum* (=typicum)

In the case when in the period of inland waters the spiked and row crop cultures of the deeply-situated soils are durably covered by water, both the culture and weed species die out. Plough-land mud vegetation develops in their place. Although it is in tight relationship with the mud vegetation found along the river, the *Cypero-Juncetum,* it can well be distinguished from that (BODROGKÖZY, 1958; PIETSCH, 1965, 1973).

Regarding its sire conditions its soil is watering character. Since the gathered rainwater washes together a large amount of colloid fraction from the neighbouring areas, the mud fraction is prevalent. During the process of drying out, mosaic-like crackings are formed on the surface.

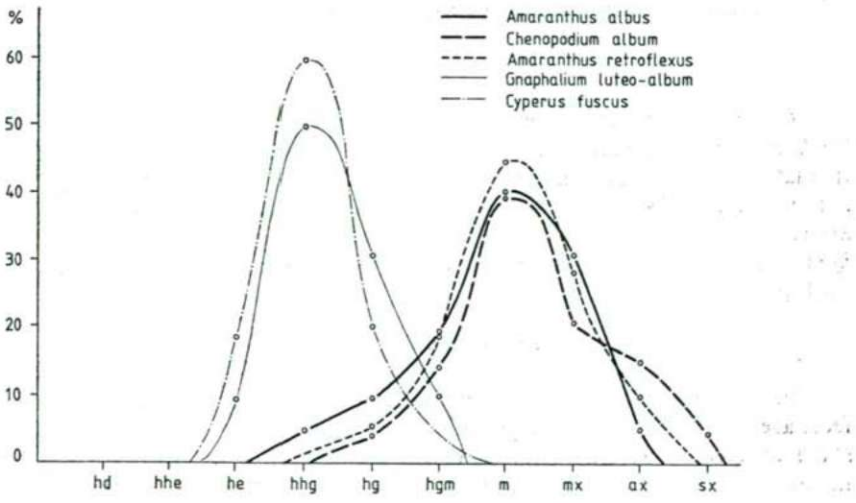


Fig. 10. Graph of the weed association species of damp-typed hard soiled spiked cultures. Evaluations are according to the H-categories.

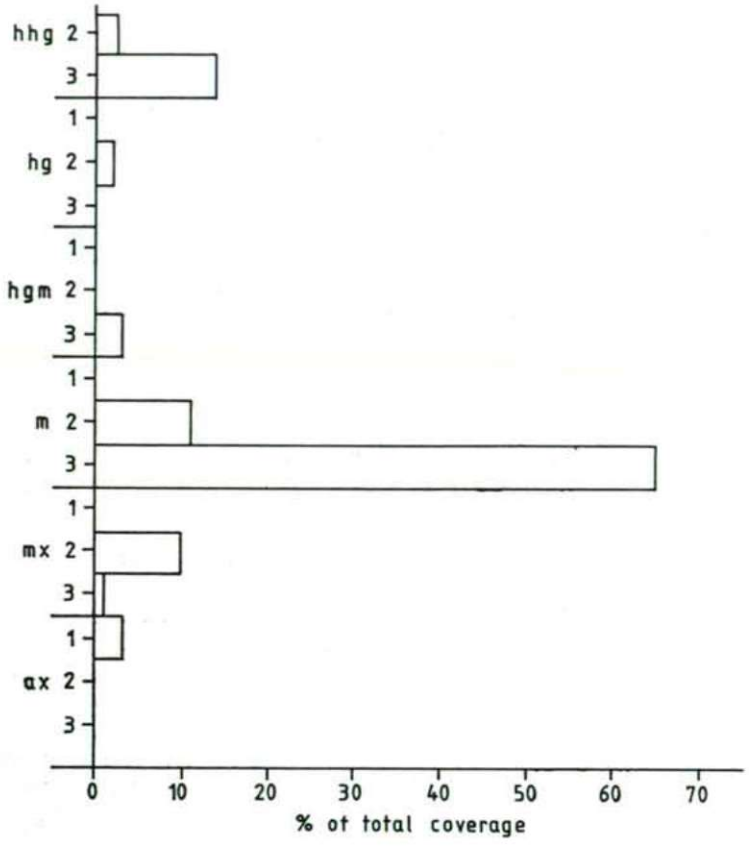
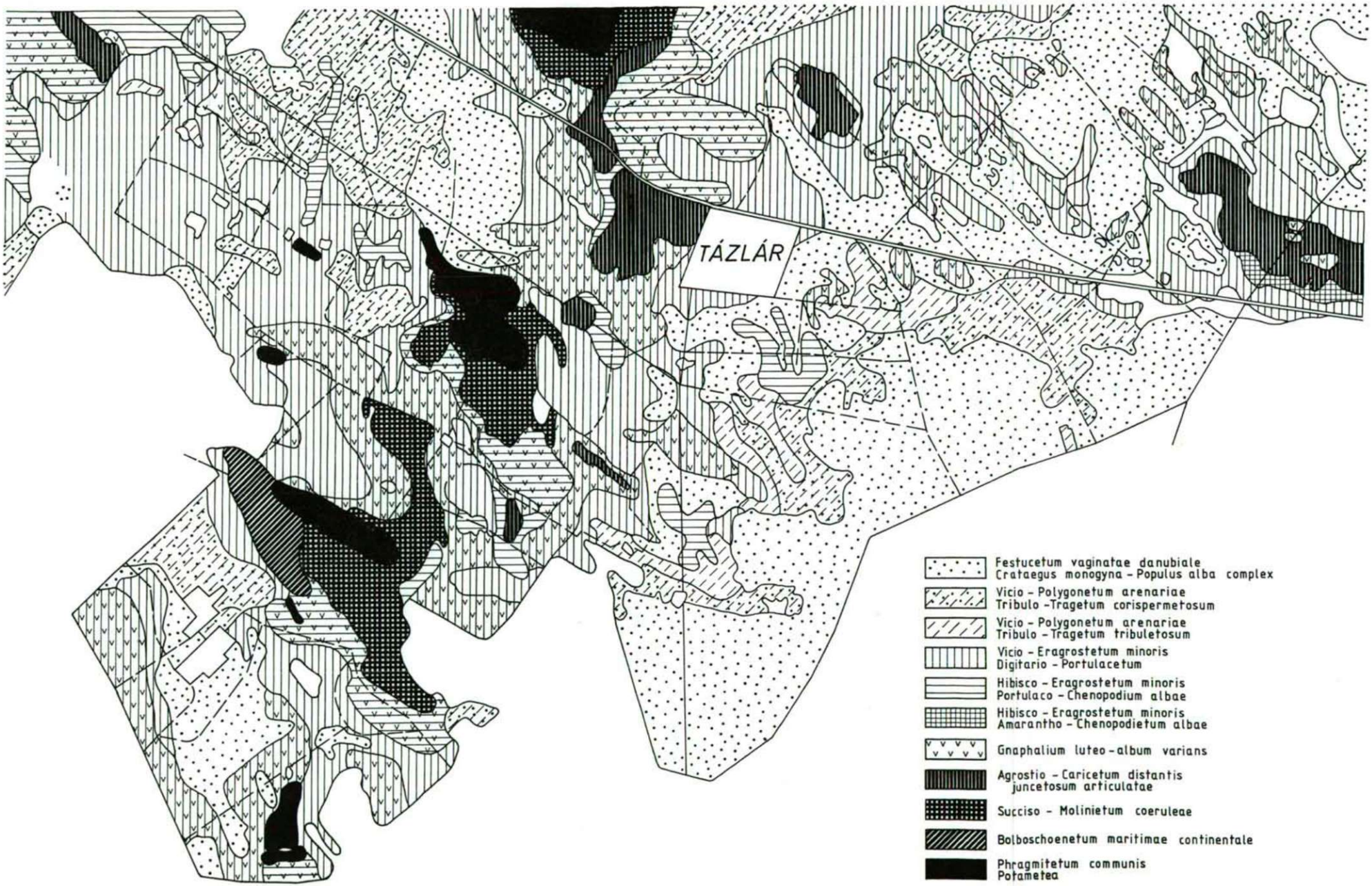


Fig. 11. Graph showing the distribution of *Amarantho-Chenopodietum gnaphalietosum* according to the total covering quota, within the H-categories.

Among the species components, the helo-hygrophytons are dominating; mainly the *hhg 2*-type *Gnaphalium luteo-album, Cyperus fuscus* and the *Pycreus flavescens*. In this latter case the culminative point of the H-curve is especially high. The *Enhinochloa crus-galli* of *hg 2* has a significant covering quota. Though it is firstly the species appearing in masses in rice fields, due to its wide H-compliance, it may also occur in the *hgm*-category as well as in the *m*-category as garden weed. Further details are given on Fig. 12.

9. *Agrostio-Caricetum distantis juncetosum articulatae*

In the areas where there are permanent inland waters and the agrotechnical effects are missing for a longer period, a more competetive perennial marsh-vegetation starts to develop. As pioneers, the characteristic representatives of the Agropyro-Rumicion crispi begin to expand, firstly the *Agrostis stolonifera* and the *Potentilla anserina, P. reptans, Trifolium fragiferum*. The appearance of *Carex distans* is followed by the *Juncus articulatus Carex serotina*, which can be held as a differential species.



Vegetation map of the area of Tázlár village, showing the conditions of extreme inland water periods when certain stands of the *Digitario-Portulacetum* were also flooded.

It has slightly szoloncsak meadow sand soil. The explored soil segment is uniform grayish-brown muddy sand till 55 cm, which means the genetic A-level. The lower B-level is made up of whitish light-grey lime carbonate sand, with occasional dark mud streaks. In the lower layer the accumulation of total salts can be demonstrated, in an amount surpassing the lowest point of the saline degree (0.015 %). This explains the appearance of *Plantago maritima, Taraxacum bessarabicum, Lotus tenuis* among the species components, which are otherwise also characteristic of *Agrostio-Caricetum.*

If these watery areas are drained, they gradually dry up and the previous species being more and more at a disadvantage are replaced by *Festuca pseudovina, Cynodon*

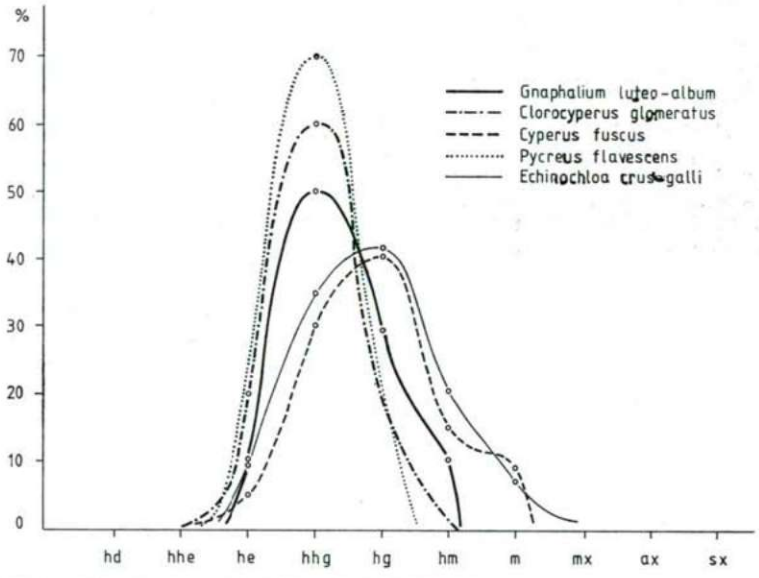


Fig. 12. H-curves of the species components of mud vegetation.

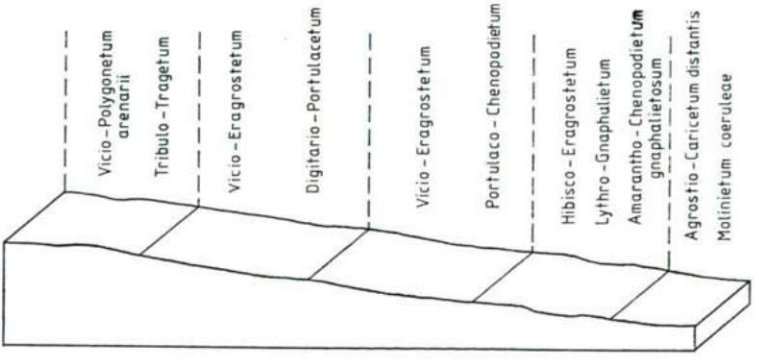


Fig. 13. Zonation system of the culture phytocenoses occurring in the studied areas, on the basis of the different reliefs.

dactylon, Cichorium inthybus, Scorzonera cana. If it is further dispensed from the devasting effect, then gradually the variant of *Potentillo arenariae — Festucetum pseudovinae Plantago maritima* may develop.

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Address of the author:
DR. GY. BODROGKÖZY
Department of Botany, A. J. University
H-6701 Szeged, P.O. Box 428
Hungary

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CORRELATIONS BETWEEN THE ZONATION OF SANDY GRASSLANDS AND THE PHYSICO-CHEMICAL CONDITION OF THEIR SOIL IN BUGAC

L. KÖRMÖCZI

Department of Botany, Attila József University, Szeged
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Abstract

Investigations were performed on the conditions of the soil of five sandy grassland communities within of the Kiskunság National Park in Bugacpuszta in 1981. The basis for comparison was the different relief conditions of the plant communities. The soil of the higher relief communities contained a higher percentage of coarse sand, in the soils closer to underground water the ratio of the colloid granules increased. Accordingly, the water balance of the colloid-rich soils was more settled, the water content was higher. The water content of the deeper layers was generally lower. The humus and nitrogen contents of the soils were lower in areas of coarser sand, in every segment showing a value wich decreased from the surface down. Their seasonal changes were in relation to the precipitation conditions and the moisture-content of the soil. The chemical reactions of the soil segments was shifted in alkaline direction by their carbonic chalk-content, and alkalinity increased with depth. Treatment with chemical fertilizer resulted in the souring of the soil and the seasonal value of the chemical reaction became more fluctuating. The higher degree of colloid fraction contributed to the improvement of the water balance and nutriment supply of the soil segments, however, due to the presence of the highly saline underground water there is also a danger of salt accumulation and alkalization.
Key words: Bugac, sandy grassland, sandy soil, alkalization.

Introduction

The aim of the investigations started in 1977 in the sandy pasture near Bugac in the Kiskunság National Park was to throw light on the relations of production and the factors influencing production of the sandy grassland communities of varying relief. The cenology of the grass associations and the physical structure of their soil was reported on by BODROGKÖZY and FARKAS (1981). The paper by KÖRMÖCZI, BODROGKÖZY and HORVÁTH (1981) discussed the productive and microclimatic conditions of these associations. SZABÓ (1975) dealt with the economy of water supplies regarding sandy soils and determined that soils with a better management of water supply and an impermeable layer are suitable for afforestation. According to the studies of SZODFRIDT and FARAGÓ (1968) the level of underground water in the sand between the Danube and Tisza is at a depth of 4—5 m on sand-hill ridges, 1.5—2.5 m between the sand-hills. Underground water is deepest in the soil of *Festucetum vaginatae*, and the soil of *Molinietum coeruleae* is water-saturated to the surface.

BODROGKÖZY (1957) and SIMON and KOVÁCS-LÁNG (1964) studied the relation between sandy soils and the series of plant associations introduced there.

The present paper also deals with the correlations between the series of sandy grassland communities and the physico-chemical characteristics of their soil.

Materials and Methods

The study area is situated in Felsőbugacpuszta in the Bócsa-Bugac region of the Kiskunság National Park. The associations studied were *Potentillo-Festucetum pseudovinae danubiale* BODROGKÖZY 59, *Molinio-Salicetum rosmarinifoliae* (SOÓ 33) 57, *Lolio-Potentilletum anserinae* KNAPP 46, *Cynodonti-Poëtum angustifoliae* (RAPAICS 26) SOÓ 57, *Achilleo-Festucetum pseudovinae* (MAGYAR 28 SOÓ 45; the cenological descriptions of wich can be found in the works of BODROGKÖZY and FARKAS (1981) and KÖRMÖCZI (1982). The difference in level between the highest and lowest points was 2.8 m (Fig. 1).

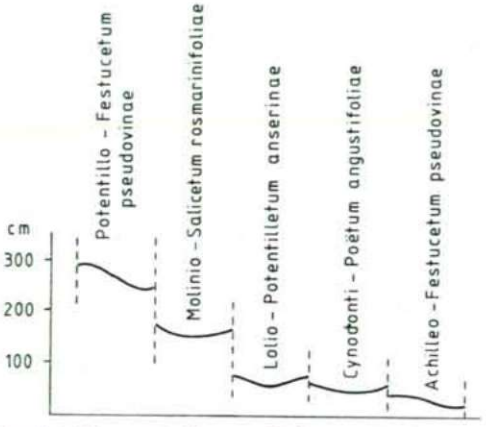

Fig. 1. Differentiation in level of the associations studied.

The meteorological data of the study year 1981 came from the measuring station in Izsák. The data are shown in a Walter-Lieth diagram (Fig. 2). Compared to the 50-year average (see KÖRMÖCZI, BODROGKÖZY and HORVÁTH, 1981. Fig. 2) the annual mean temperature was 0.5°C higher and the annual precipitation was 151 mm lower. It is characteristic of the inequality in the distribution of

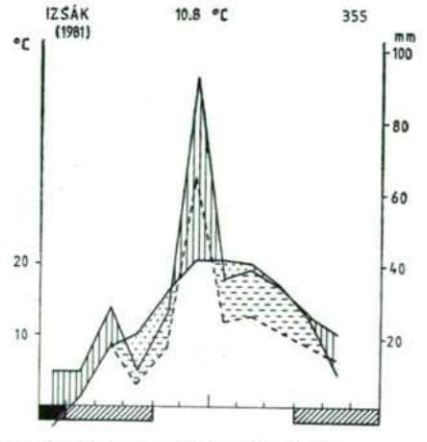

Fig. 2. Weather for the year studied shown on Walter-Lieth diagram.

precipitation that the year had meagre rainfall from March to October, with specific drought in spring and at the end of summer and a larger amount of rain (92 mm) fell only in June. The precipitation in winter was 1/3 of the average.

In 1981, from February till October samples were taken monthly from the soil of the associations with the help of an auger in layers of 10 cm.

The moisture content of the soils was calculated after drying at 95 °C and weighing the soil again. The water content was determined as a percentage of the weight of the damp soil.

The physical composition of the soil samples was measured by the sedimentation-hydrometer method, and the hygroscopy (hy) was also measured (BALLENEGGER 1953).

The $CaCO_3$ content was measured with a Scheibler apparatus. The humus-content was measured by using the permanganometric method, and the total nitrogen content was calculated with an ammonia selective electrode after Kjeldahl-destruction.

The determination of the total water soluble saline content was carried out on the basis of conductivity.

Distilled water extract was used for the hydrogen ion concentration measurements.

Results and their evaluation

Of the five associations studied the *Potentillo-Festucetum pseudovinae danubiale* BODROGKÖZY 59 pasture grass was from the areas of highest relief, the soil of wich is coarse humous sand. The ratio of granules larger than 0.4 mm was 3—4%, that of granules between 0.1—0.4 mm was 75—87%, the washable fraction was 7—15%. The hygroscopy (hy) of the soil varied between 0.64—0.15 according to the depth. The higher value at the surface was a result of the humus content. The $CaCO_3$ content of this soil was low, in the 0—30 cm surface layer no chalk content was demonstrable and even in the deeper layers it was only between 3—7%. Accordingly, the chemical reaction of the diluted extract was also low.

The water content of the association's soil showed inordinate fluctuation. The water content in the surface 10 cm layer expressed in a percentage of wet weight varied between 3—25%, being dampest at the end of winter, drying out gradually by August, and again becoming higher in water content by autumn. The higher precipitation in June (92 mm) only increased the water content of the deeper layers to a small extent, and only delayed the fast drying up of the surface layer. The water content of the deeper layers was lower than that of the surface layer, the effect of the summer precipitation prevailed downwards, with a shift in time.

The humus content of the upper 20 cm soil layer was relatively high (3—4.5% and 1.5—3%, respectively), that of the lower layers did not reach 1%. The rather high value measured in February (7.4%) was obtained from the phytomass of the previous year, which later decomposed rapidly with the drying of the soil. The decomposition of the humus material was rather fast; the humus content in the surface soil layer showed rather great fluctuation, which was caused by the continuous replacement of leaf-mould. A more intensive summer humus-formation was observable in the 10—20 cm layers, which coincided with the period when there was a lack in precipitation.

The seasonal changes in the total nitrogen content were similar to those of the humus content. It showed a gradual decrease throughout the whole vegetative period, with a slight increase in the middle of summer. Its value in the surface layer ranged from 0.8—2.0 mg/g, and was 6.2 mg/g at the beginning of the vegetative period.

The chemical reaction of the soil showed great fluctuation, shifting to alkaline from the surface down. The chemical reaction of the surface layer decreased from 7.8 to 5.6 pH by the middle of the summer, then rose gradually until it became neutral. The chemical reaction of the deeper layers varied from 7—9 pH, their course differed, being the reverse in the middle of the vegetative period, as in the surface layers. The souring of the soil was presumably due to the vegetative period (ALMÁSSY et al. 1968).

Water soluble salt was not measurable in the soil of the *Potentillo-Festucetum pseudovinae* association.

The *Molinio-Salicetum rosmarinifoliae* (Soó 33) 57 is an association of higher situated wind furrows, probably developed from the association of *Schoenetum nigri-*

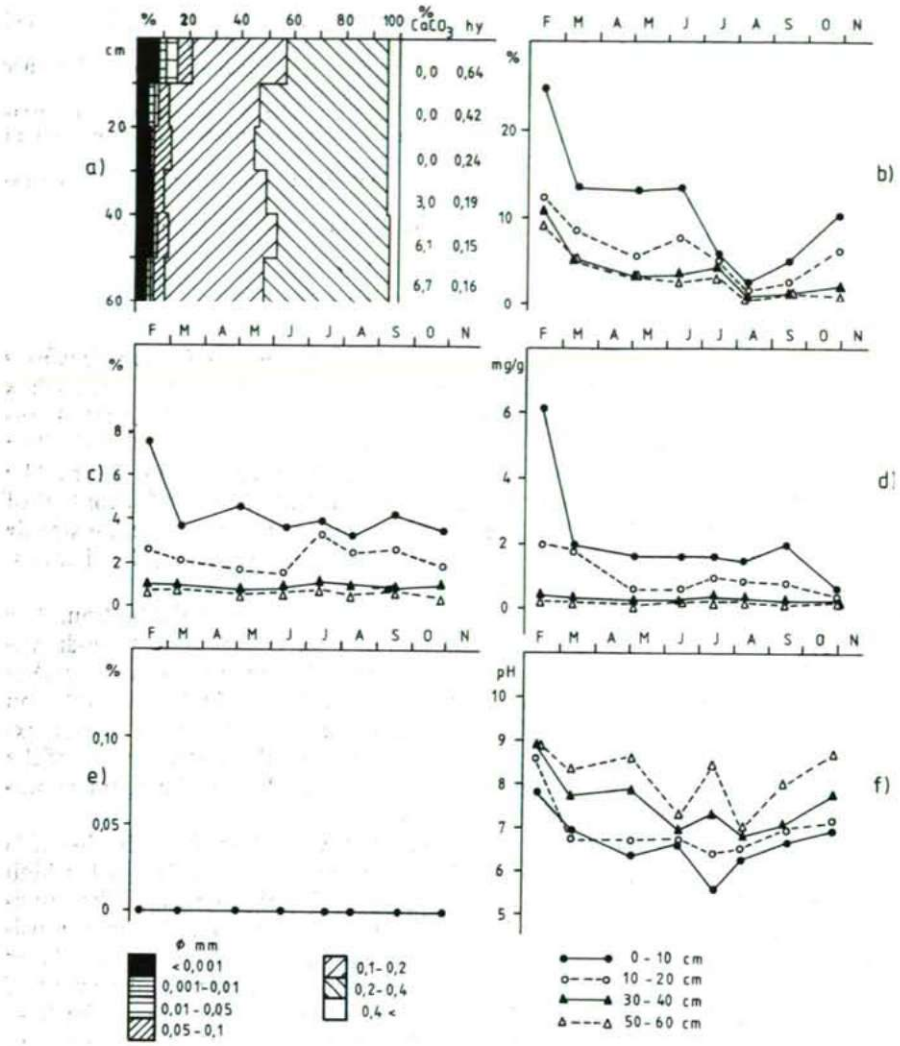

Fig. 3. Results of analysis in the *Potentillo-Festucetum pseudovinae* association. Physical composition of the segment, $CaCO_3$ content and hygroscopy (a); moisture content (b); humus content (c); total nitrogen content (d); water soluble salinity (e); and hydrogen ion concentration (f.)

cantis (ALL. 22) W. KOCH 26 from a damper site, as a consequence of the gradual drying up of the area (BODROGKÖZY and FARKAS, 1981).

The association has fine granular soil, made up of averagely humous sand, in the granular composition of which granules having diameters of 0.1—0.4 mm dominate. The ratio of the granules with diameters of 0.2—0.4 mm was 25—35%, the 0.1—0.2 mm granules had a ratio of 37—48%. The washable fraction was between 2—7%. The hygroscopy (hy) of the soil segment varied from 0.52—0.17. The $CaCO_3$ value measurable in the soil segment decreased from the surface to 40 cm, then greatly increased, being 16.1% at 60 cm and 20.6% at 70 cm. The moisture content of the soil

was low throughout the whole study period, its value decreased downwards from the surface, not even reaching 15% in the dampest upper layer. The fluctuation of the water content was the greatest in this layer, ranging from 3—13%. Due to the proximity of the underground water level the water content was again higher in the 50—60 cm layer, showing a value of about 10%, with only slight fluctuation.

Excluding the upper 10 cm layer, the organic matter content of the soil segment was low, having a value under 1%. Fluctuation was slight, showing a small rise at the beginning of the vegetative period. The humus content of the upper 10 cm layer varied from 1.8—3.7%, gradually increased in spring, fell back when the soil layer dried out and increased again in the second half of the vegetative period.

The seasonal dynamics of the nitrogen content was similar, however, the second maximum value was observed later and the decreases were steeper. The double maximum of the nitrogen content was observable in the whole soil segment. Its value ranged from 0.7—1.5 mg/g on the surface, and from 0.1—0.4 mg/g in the lower layers.

The chemical reaction of the segment was neutral-slightly alkaline during the whole vegetative period, its hydrogen ion concentration varied between 7.2—8.8 pH. The fluctuation of the chemical reaction was slight in the whole soil fragment, within 0.3 pH, and the changes occured in parallel. The hydrogen ion concentration of the upper 10 cm layer was more acidic than that of the lower layers, and its fluctuation reached a value of 1 pH.

Water-soluble salts were not demonstrable from this segment either.

In the spaces between the sand hills, which are damper and closer to the underground water level, is situated the association of *Lolio-Potentilletum anserinae* KNAPP 46, the soil structure of which significantly differs from that of the previous association; it has a better management of water supply, therefore making possible the establishment of more particular species, too (KÖRMÖCZI, 1982).

The physical composition of the soil segment is characteristic of the fact that in the upper 30 cm layer the ratio of the washable fraction is high (33—41%), but is only 15% in the layers between 30—40 cm. This latter layer is a transition towards the skeleton soil of the lower level, which is coarse sand with a washable faction of 8%. The ratio of granules larger than 0.1 mm was 47—57% in the upper layer, 76% in the transitional layer and 89% in the lower ones. In compliance with the physical structure the hygroscopy of the upper level of the segment was also high (hy 1.88—0.54), but was low in the deeper layers (0.19—0.10).

The segment could be devided into the afore-mentioned two levels also on the basis of the $CaCO_3$ content, which was 20—22% the upper level and 3—7% in the lower one. The water retaining capacity of the upper soil level was rather good, ensuring an appropriate water supply for the plants all through the year. The initial 43% water content did not fall below 25% even in the drought period. The subsoil moisture content of the coarse sand was also high, nearing a value of 20%, however, this was a result of the proximity of the underground water level. With the decrease in the level of underground water, the water content of the lower layers also decreased gradually, reaching 8% by the end of the study period.

The humus and total nitrogen contents of the soil segment were high. The humus content varied from 4—10% in the upper level, but was around 1% also in the deeper layers. In the early summer period when there was a lack of precipitation there was a significant decrease in the humus content, nevertheless, it was gradually replaced by autumn from the large quantity of phytobio-mass. In the 10—20 cm layer the organic matter content decreased further, but was of a lesser degree. This change in the humus content of the deeper layers was not similar to that of the upper layers.

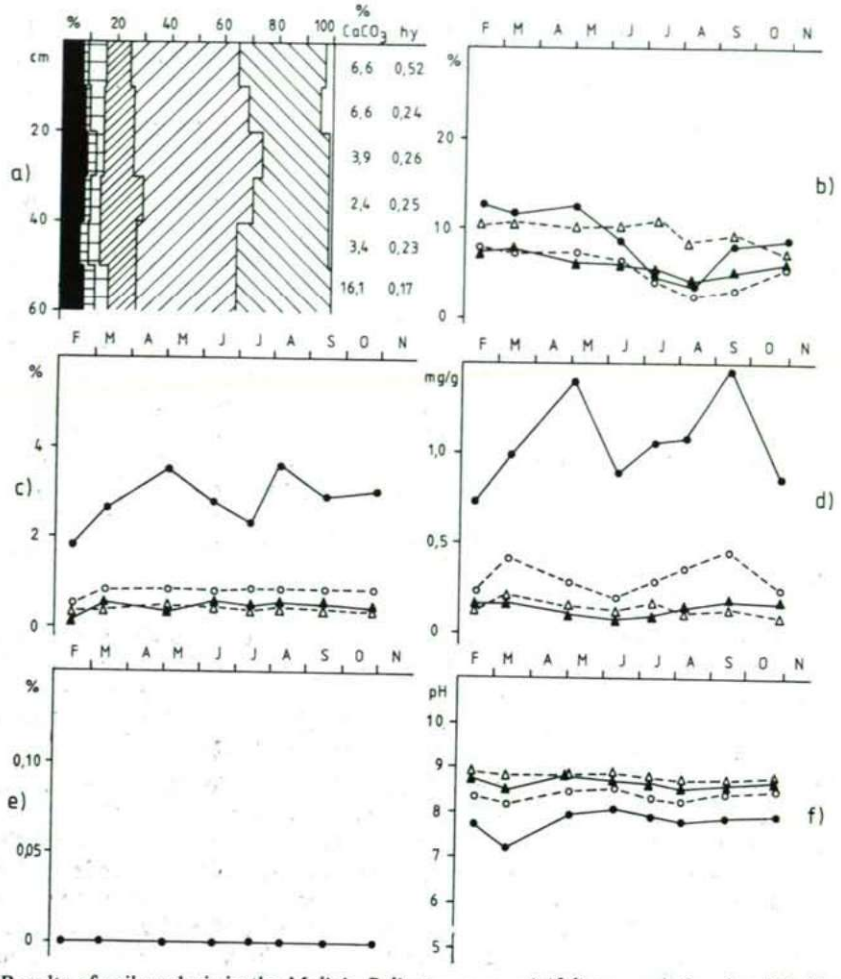

Fig. 4. Results of soil analysis in the *Molinio-Salicetum rosmarinifoliae* association (see labellings for Fig. 3).

The value of the total nitrogen content was 3—5 mg/g in the 0—10 cm layer, 2—3 mg/g in the 10—20 cm layer and under 1 mg/g in the deeper layers. Its change in the upper 10 cm layer was similar to that of the humus content, decreasing significantly in the period when there was a lack of precipitation by 1.3 mg/g, and rising by the same amount gradually by autumn. The chemical reaction of the soil segment was slightly alkaline, with values between 7.5—8.5 pH. Going downwards the alkalinity increased. The pH value did not show significant seasonal changes.

A large amount of water soluble salt was measurable in the upper 40 cm layer of the soil segment. Here the Na salts dissolved in underground water accumulated due to the upward flow of the underground water and the higher colloid granule content of the upper soil layer, involving a danger of alkalization. The highest salt content was observable throughout in the uppermost soil layer, moving within the limits of the saline value (0.1 %). In the period lacking precipitation the salt content of the soil increased, showed a decrease in the second half of the vegetative period, and was

completely washed out of the 30—40 cm layer by the end of the period, falling beneath the saline value even in the upper layer. Water soluble salt was not detectable in the subsoli (40—60 cm).

In one part of the area of the previous association the upper soil level was disrupted and removed to a depth of 25 cm. With the reestablishment of the plants an association of *Cynodonti-Poëtum angustifoliae* (RAPAICS 26) SOÓ 57 developed in this area, the soil of which had a bad water and nutriment supply despite the proximity of the underground water level, as a consequence of the small basal area of the vegetation.

The soil of the association was coarse sand, where the ratio of granules with diameters of 0.2—0.4 mm was 28—55%, that of granules having diameters of 0.1—0.2 mm was 37—48%, and the ratio of washable areas was 9—11%. Going downwards the ratio of coarse granules showed an increase. The hygroscopy of the soil segment varied between 0.14—0.09.

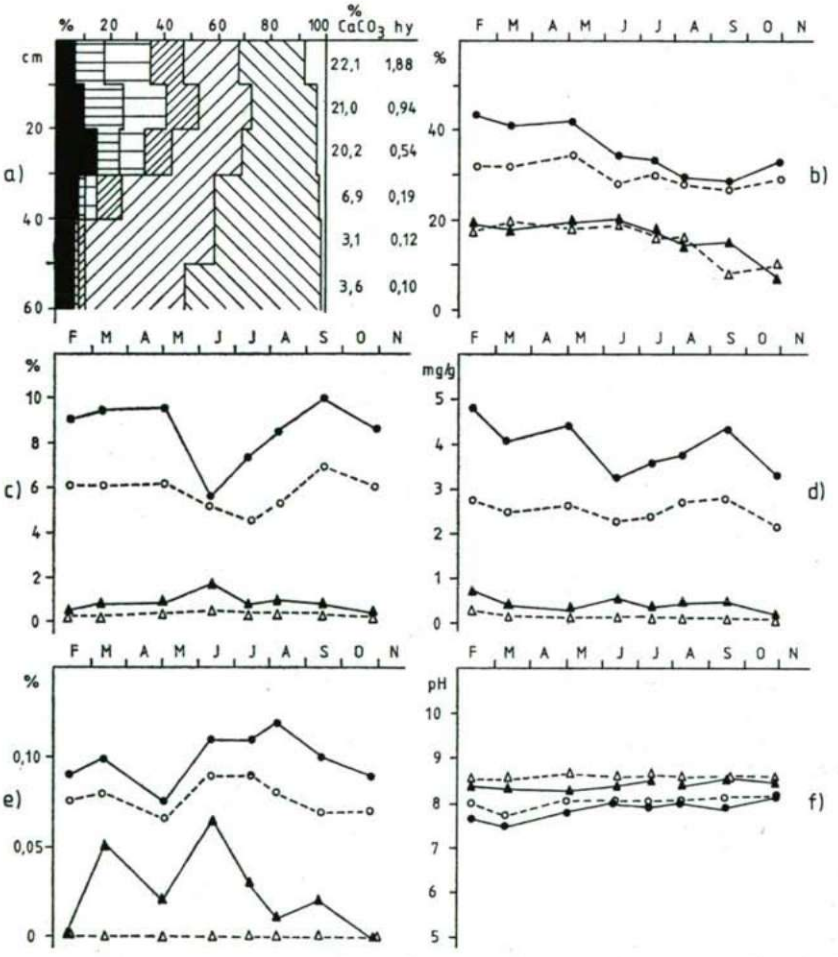

Fig. 5. Results of soil analysis in the *Lolio-Potentilletum anserinae* association (see labellings for Fig. 3).

The $CaCO_3$ content of the soil segment was found to be higher than the value measured in the lower level of the soil of the previous association, but that of the upper layer was lower. This can be explained by the washing away of the $CaCO_3$ from the upper level, since the degree of eluviation increased after the removal of the upper level rich in colloids (STEFANOVITS, 1981). The $CaCO_3$ content ranged from 8.3— 11.2%.

The water balance of the soil segment was bad, as consequence of the decrease in the underground water level the whole segment gradually dried out, the moisture content decreased to 1% by the middle of summer in the upper layers and that of the deeper layers also fell beneath 5%. By the end of autumn a moisture content of about 5% was measured in the whole segment.

The humus content was low during the whole period, not reaching 1% in the 0—10 cm layer, and being under 0.5% in the lower layers; ranging from 0.2—0.5%. By August a slight increase could be experienced in regard of the humus content. The total nitrogen content developed similarly to that of the humus; the nitrogen content of the layers under 10 cm varied only slightly, and that of the 0—10 cm layer was twice that of the lower ones. Its value was rather low, 0.18—0.34 mg/g in the upper layer and between 0.04—0.17 mg/g in the deeper ones. A slight increase could also be experienced in this respect by the end of summer.

The hydrogen ion concentration of the soil segment was slightly alkaline, falling between 8.2—9.1 pH and becoming slightly more alkaline further down. The pH-fluctuation of the various layers was within 0.3. No water soluble salts were demonstrable from the segment.

Of the associations studied the *Achilleo-Festucetum pseudovinae* (MAGYAR 28) SOÓ 45 was situated at the lowest relief, where the process of alkalization was detectable. The upper part of the soil of this association (till 50 cm) was sandy adobe, turning to coarse sandy subsoil in the lower layers. The ratio of granules with diameters of 0.2—0.4 mm was 21—30%, that of granules with diameters of 0.1—0.2 mm was 26—35%. The ratio of washable areas was 21—37%. The hygroscopy of the segment ranged from 0.60—0.11. The $CaCO_3$ content was found to be rather high; between 20.8—34.4%. Because of this the colour of the soil was whitelight grey. This also significantly shifted the chemical reaction in an alkaline direction; values of about 10 pH were measurable. (The hydrogen ion concentration varied from 8.3 to 10.4 pH). The $CaCO_3$ content increased from the surface to a depth of 40 cm, and below from this it decreased again. The chalk-content of the different layers had a positive correlation with the ratio of colloid granules.

The changes in the moisture content of the various layers showed variability. In the surface layer of 10 cm the water content stayed at the same level till the middle of summer — at a value of 17—18% —, then it suddenly decreased to 7% and gradually increased again by autumn. The water content of the 10—20 cm layer gradually decreased from 20% to 8% from spring till the end of summer, and the increase following this was also gradual. The value and change of the water content was slighter, too, in the lower layers; values of 8—12% were measured in the 30—40 cm layer. In conformity with the gradual decrease in the level of underground water, the water content of the lower levels also showed a constant decrease. The upper part of the soil segment was rich in humus. A gradually increasing humus content between the values of 1.9—4.7% was measurable in the 0—10 cm layer. The humus content of the 10—20 cm layer ranged from 0.8—3.1%, showing great fluctuation. In the lower sites the humus content did not reach the value of 1%. As in the humus the value of the total nitrogen content increased slightly until autumn from 0.9 mg/g to 1.9 mg/g, however, its va-

lue fell to half in the 0—10 cm layer at the end of autumn. The nitrogen content also showed great fluctuation in the 10—20 cm layer, being between 0.3—1.2 mg/g.

A high, water soluble total sa linity, well above the saline value, was measured in the soil of the *Achilleo-Festucetum pseudovinae* association. The total salinity of the whole segment was above 0.1 % till the middle of summer. The highest value was measured in spring — 0.38 % — rom which value it gradually decreased till the end of the study period. By the time of the last sampling soluble salt could not be demonstrated at all in the whole segment. The soluble salt content of the segment decreased together with that of the underground water level, and the salts were gradually washed out with the precipitation. Due to the coarse sand composition of the subsoil there was no possibility for the replacement of the salts from the underground water, therefore the salt content was probably washed out completely from the segment.

The correlations between the humus- and salt content need further study.

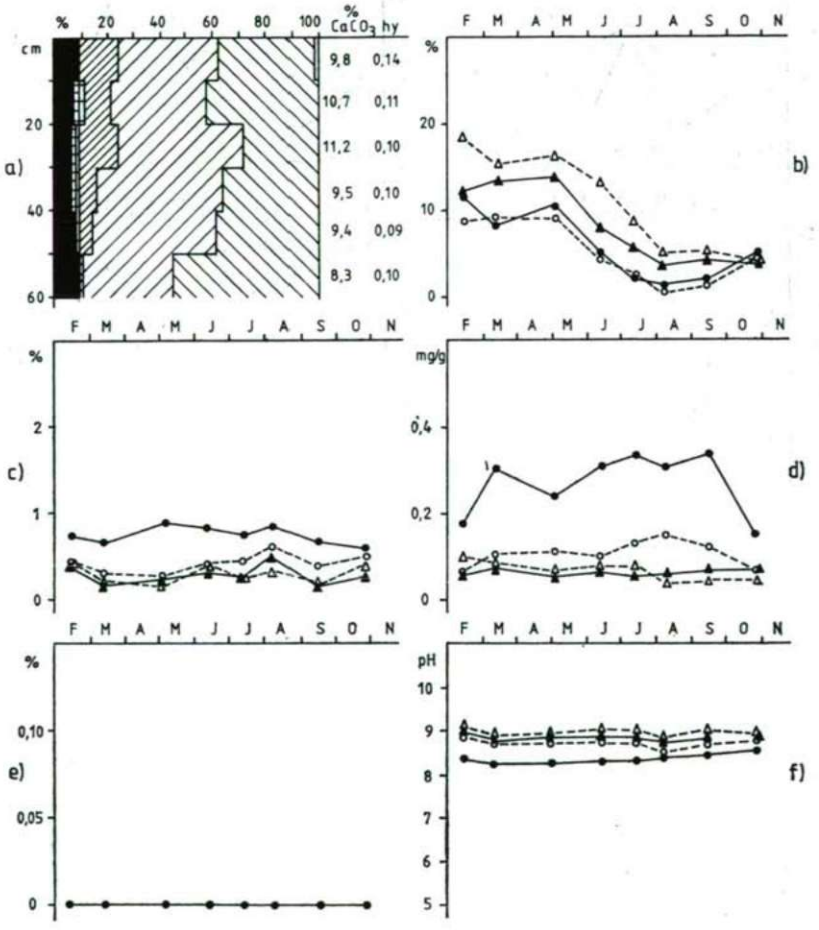

Fig. 6. Results of soil analysis in the *Cynodonti-Poëtum angustifoliae* association (see labellings for Fig. 3).

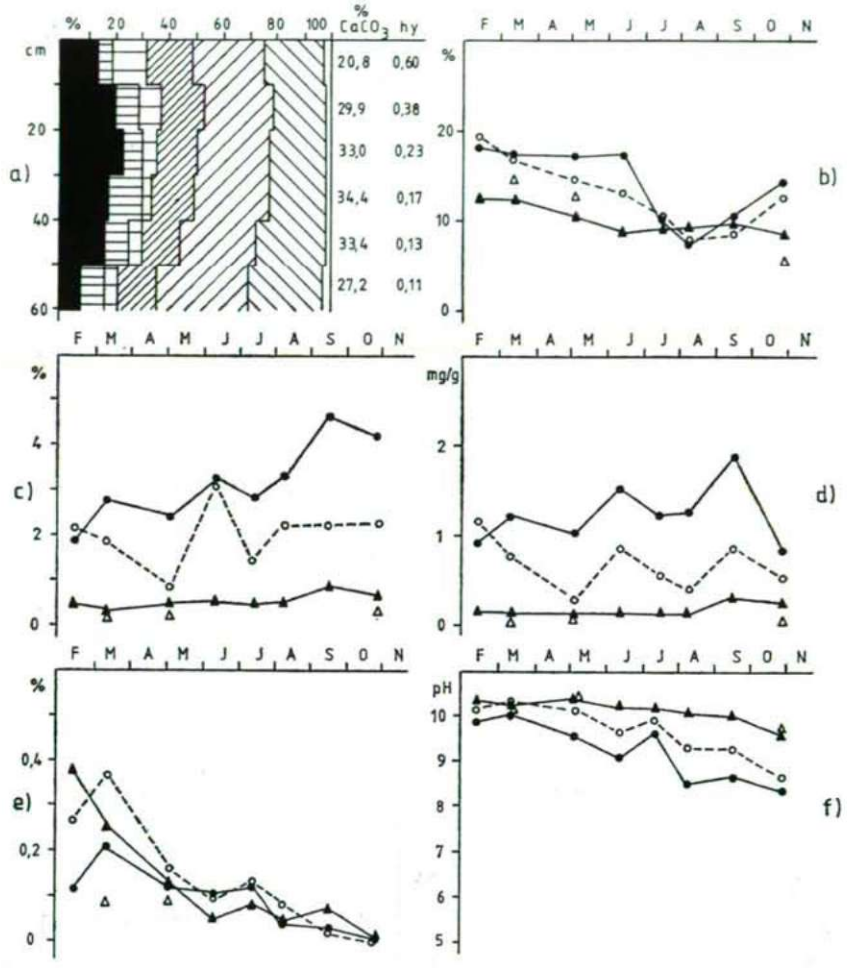

Fig. 7. Results of soil analysis in the *Achilleo-Festucetum pseudovinae* association (see labellings for Fig. 3).

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Address of the author:
DR. L. KÖRMÖCZI
Department of Botany, A. J. University
H-6701 Szeged, P.O. Box 657.
Hungary

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ULTRASTRUCTURAL STUDIES ON THE GASTROINTESTINAL NERVOUS SYSTEM OF HELIX POMATIA

A. ÁBRAHÁM

Department of Zoology, Attila József University, Szeged
(Received July 1. 1982)

The gastrointestinal nervous system is made up of neurons, nerve fibres, glia cells and glia fibres The neurons are large and sharply defined towards the surroundings. They contain many endoplasmic reticules having wide lumen, as well as free ribosomes and ribosomes arranged in rows. Mitochondria and lysosomes are few in number. Neurosecretory granules are characteristic, among which dense-core forms and larger homogeneous oval granules can also be detected. The chromatin is found close to the nuclear membrane in the form of indented lobules. One part of the nerve fibres contains large amounts of neurosecretory granules the other part is prevalent in the dense-core forms. There are added to the nerve fibres of granule content, the agranular fibres containing no granules, as well as glia cells and glia fibres. Many glycogen granules can be found in the latter two. Their characteristic components are the gliogranules.
Key words: gastrointestinal nervous system, *Helix pomatia.*

Introduction

The intestinal canal of *Helix pomatia* begins at the orifice which is on the ventral side of the head between the two labium tentacles, and leads into the wide pharynx. The short oseophagus opens from here and continues in to the brownish stomach. On this lies the two flattish salivary glands, which cower a large area lenght-wise and widthewise adhering to the dorsal side on a short section. Both go through a ribbon-like, twisted canal, which protracts on both sides of the oesophagus and leads into the pharynx (ÁBRAHÁM, 1939, 1966, 1967). The stomach is followed by the small intestine which after a short spiral course, enters into the bend of the visceral bursa. Here it turns to the other side and in the form of a dilation resembling the rectum it opens to the outside on the inner side of the lung cavity (ÁBRAHÁM, 1969; SCHMALZ, 1914; THOMAS, 1951).

Materials and methods

Small pieces were cut from the oesophagus, stomach and intestine of the animals narcotized with chloroform and material were fixed with 0.5% osmium acid following prefixation with glutaraldehyde and embedded in araldite after the usual dehydration. Ultrathin sections were prepared by means of an LKB ultramicrotome and examined under TESLA D 242 and JEOL B 100 electronmicroscopes. The studies were carried out in the Biological Research Institute of the Hungarian Academy of Sciences (Tihany), the Central Research Institute for Medicine of the Hungarian Academy of Sciences (Budapest), the Ist. Institute of Anatomy of the Semmelweis Medical University (Budapest), and the Electronmicroscope Laboratory of the Central Research Institute for Biology of the Hungarian Academy of Sciences (Szeged). During the course of our studies we received help from DR. I. BENEDECZKY, DR. D. SZABÓ, DR. J. HÁMORI, DR. F. JOÓ, and DR. IDA TÓTH, to whom we should also like to express our sincere thanks here.

Results

The structure of the neurons showed a strange and irregular appearance. Many neurons here been observed by electronmicroscopy, but the author has not seen any similar to these in his own pictures, nor in the literature (SCHARRER and BROWN, 1961; SCHARRER, 1963; KUHLMANN, 1963; SANCHES and BORD, 1958.) The peculiarity, however concerned the cytoplasm; the cell nucleus showed the general and usual neuron nucleus form.

The large amount of endoplasmic cysternae was striking in the cytoplasm (SCHLOTE and HANNEFORTH, 1963). The ribosomes arranged in rows were observable on the boundary of these. Nevertheless, it should also be mentioned that the whole cytoplasm was almost overrun by free ribosomes. The mitochondria belonged to the crystae type their number was relatively low. Many neurosecretory granules of various situations and forms were found in the cytoplasm. Most of them were roundish and smooth, which is the usual form for neurosecretory granules. Nevertheless, dense core forms were also detectable, although extremely rarely. As in the brain of the water beetle (ÁBRAHÁM, 1966, 1967, 1969), (Dytiscus marginalis) there were many double and triple groups among the smooth granules. The single groups were circumscribed by capsules. Multivesicular bodies laden with small roundish vesicules were not infrequent in the cytoplasm. The greater part of the nucleus was empty. The chromatin adhered to the nuclear membrane in the form of dense nodules and roughly indented laminae. The two nuclear membranes were found to be uniform laminae the nuclear membrane pores were not observable (Fig. 1).

Among the forms of nerve fibres those found in greatest number contained granules. The latter usually filled the axoplasm completely. Among these there were some which were roundish and elyptic of which some were smaller others larger. They were situated very close to the axolemma and were smooth and homogeneous. Clear vesicules were detectable in the axoplasm mainly near the axolemma, but also in other places, although in smaller quantities. The various kinds of granules were all smooth and their number was particularly high in the area of the stomach and intestine (Fig. 2).

Besides the nerve fibres completely filled with granules, especially in the wall of the intestine, some fibre forms were not infrequent where only a few granules were observable in a rather large area. Sometimes, however, oblong dilatations were detected in the course of the nerve fibres, which occurred again along the fibres. These were filled with roundish granules while no granules at all were seen in the section of fibre linking the two neighbouring dilatations. Since the fibres completely filled with granules were found in the highest quantity the phenomenon could be regarded as a functional state (JUNGSTAND, 1962; KRAUSE, 1960).

Although in smaller numbers such nerve fibres were also found in the nerve fibre plexuses in which, besides mitochondria, dense core vesicules, (or only the latter) could be detected. Their amount was usually low. The fibres containing dense core vesicules seemed to be almost empty, though mitochondria appeared in higher quantities than in the nerve fibres of the plexuses containing smooth neurosecretory granules (Fig. 3).

In the nerve fibre plexuses apart from the nerve fibres comprising neurosecretory granules and dense core vesicules, nerve fibres also occured which did not contain any kind of granules or vesicules. These fibres are called agranular nerve fibres. On our pictures these are clear formations differing entirely from the other form of fibre. The axolemma was well defined, neurotubules appeared, in large quantities and with

Fig. 1. Detail of neuron from the intestinal wall of snail. The karyoplasm of the nucleus (N) is light, few electron dense heterochromatin are situated along the cell membrane. Neurosecretory granular vesicules (g), wide, rough surfaced endoplasmic reticulum cysternae (rEr) and many ribosomes (r) can be seen in the cytoplasm. The Golgi apparatus (G) is present in the form of concentric sacs. M = mitochondrium, 1 = lysisome, Sm = smooth muscle cell, Gl = glia process X 20 000.

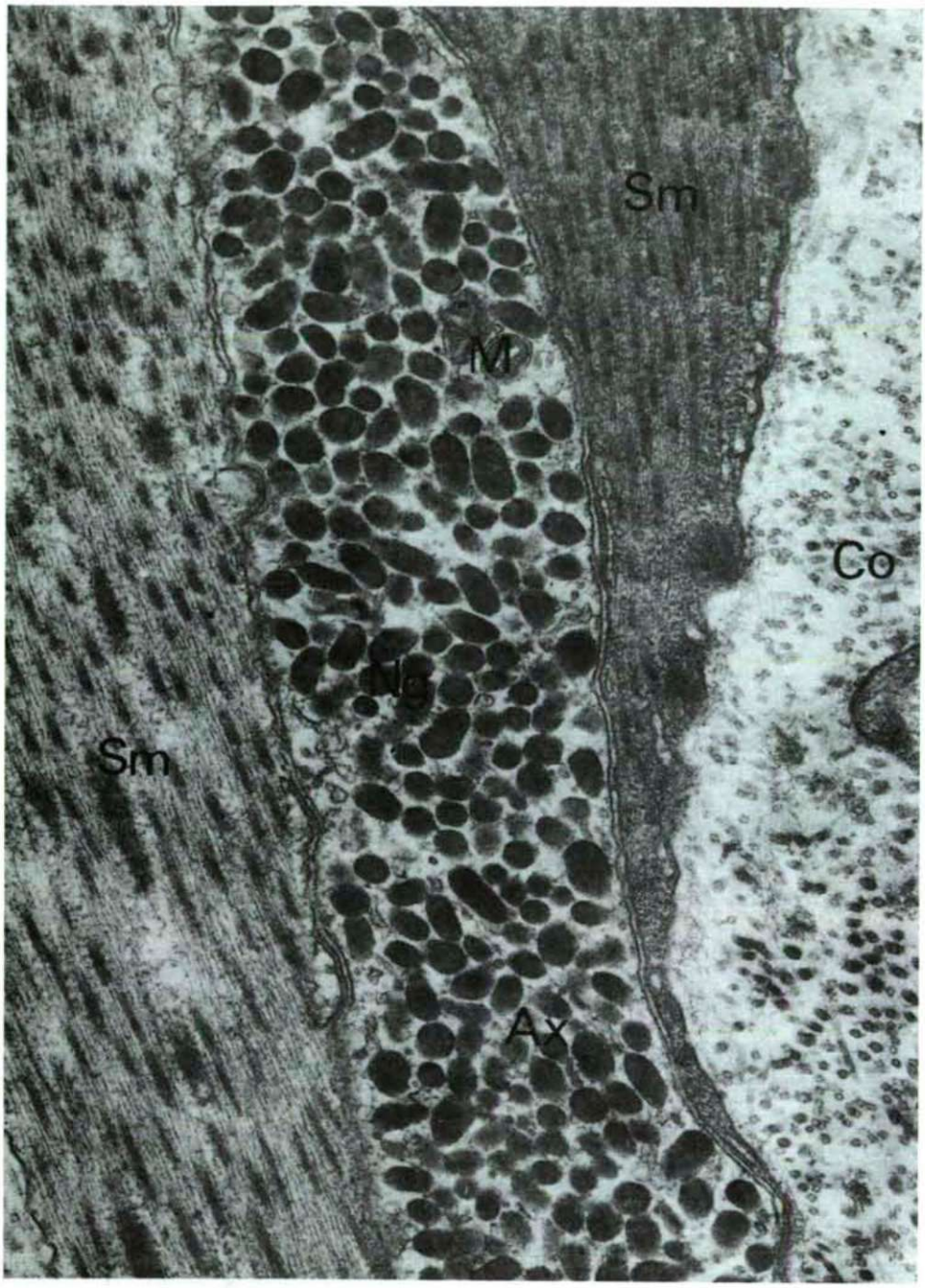

Fig. 2. *Helix pomatia:* nerve fibre from the intestinal wall. Ng = neurosecretory granule, Ax = axon, M = mitochondrium, Sm = smooth muscle, Co = collagen fibres X 20 100.

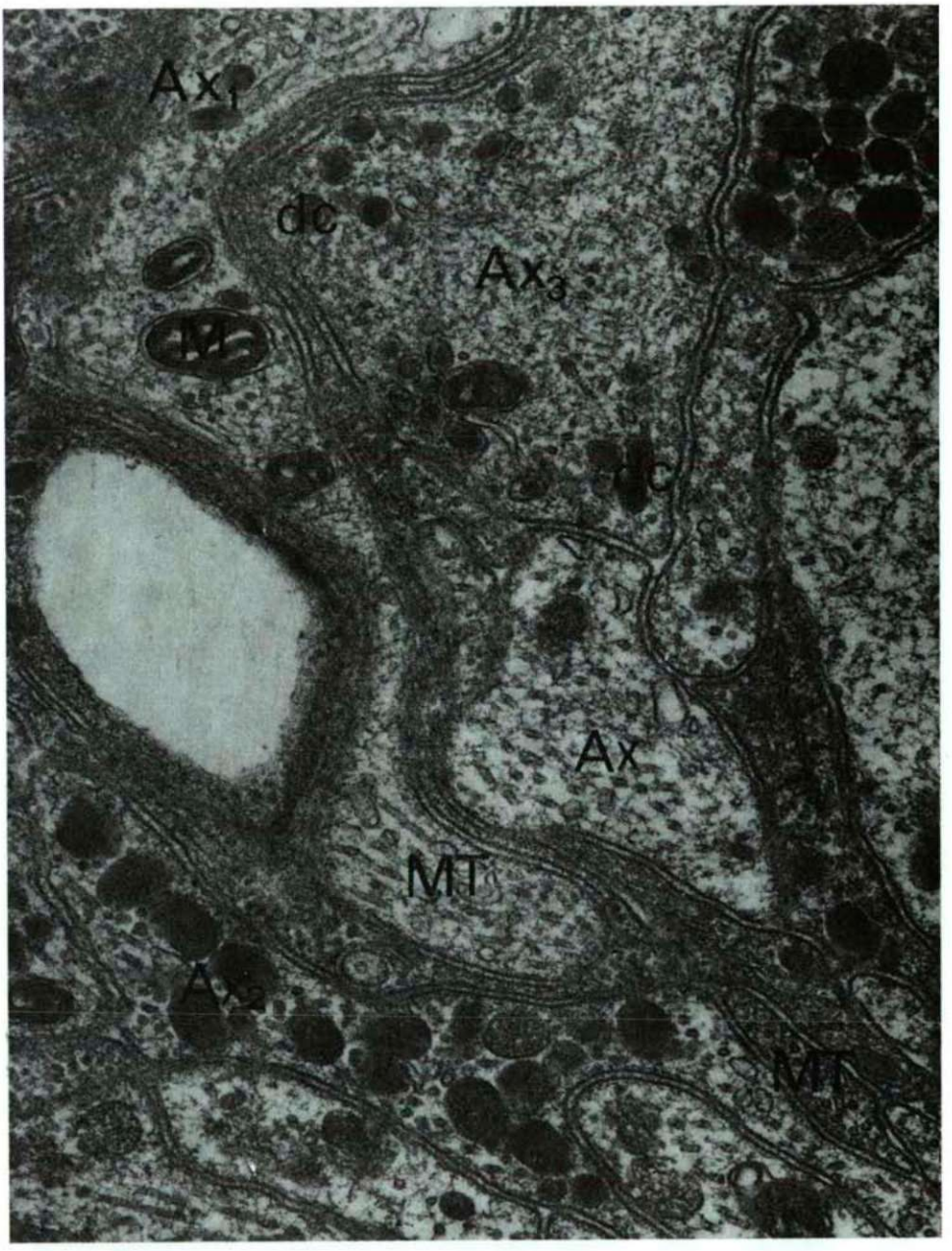

Fig. 3. Cross section of nerve fibres (Ax) in the nerve plexus of snail intestinal wall. In some axons (Ax_1) one or two mitochondria can be seen apart from the microtubules (MT). In other axons (Ax_2) electron dense neurosecretory granules and (Ax_3) dense core vesicules (dc), are observable. (X 48 000.

Fig. 4. Agranular nerve fibres from the wall of the intestine. Ax_1 and Ax_2 = axon cross sections, Mt = microtubules, Co = collagen fibres, Sm = smooth muscle, Gg = gliogranules, g = glycogen granules. X 21 100.

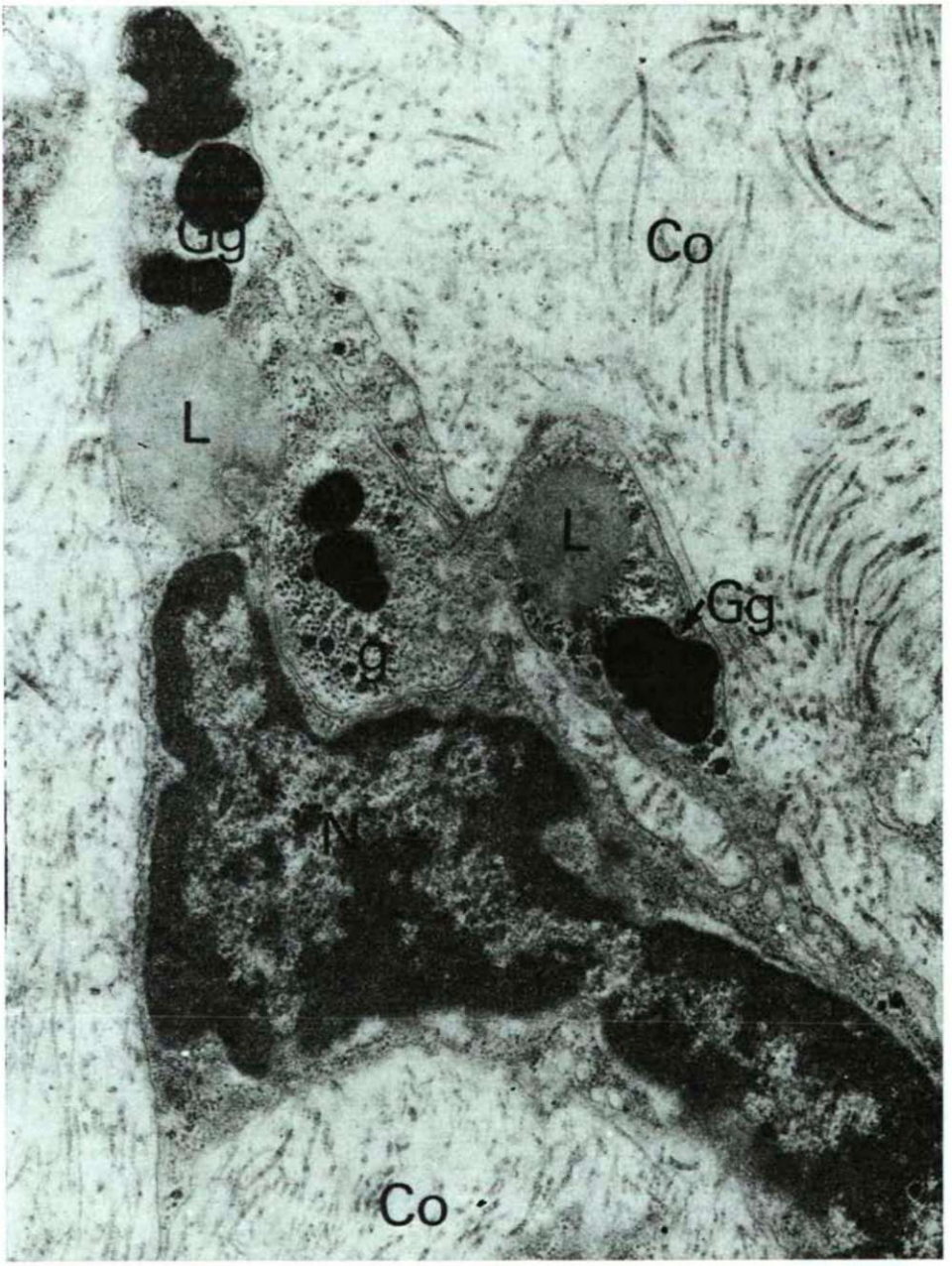

Fig. 5. Glia cell from the intestinal wall of snail. Many heterochromatin can be found in the processing cell nucleus (N). Lipid droplets (L), gliogranules (Gg), glycogen granules (g) and collagen fibres (Co) X 25 000.

unusual sharpness in the axoplasm, both in longitudinal and cross sections. Just the same as the fibres themselves the neurotubules were also very clear. In low number and small areas irregular cavities were observable in the axoplasm. These are held to be the cysternae of the endoplasmic reticulum (Fig. 4).

The neuron and nerve fibres were surrounded by processing glia cells (SCHLOTE and HANNEFORTH, 1963). The cytoplasm was found to be filled with glycogen granules. The thick processes were seen to depart from cells with wide bases, and in the majority of cases they accompanied the nerve fibres in the form of fibres. The cell nucleus was strongly elongated, the chromatin formed large nodules centrally. The glycogen granules and gliogranules were the components of the glia cells and glia fibres. The glycogen granules were polyedric and completely filled the cytoplasm. The gliogranules were homogeneous roundish or ellipsoid small infractions and indentures were observed on some of them. At times they were seen interlaced with each other (Fig. 5).

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Address of the author:
PROF. DR. A. ÁBRAHÁM
Department of Zoology, A. J. University,
H-6701 Szeged, P.O. Box 659.
Hungary

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ULTRASTRUCTURAL STUDY OF THE FORMATION OF SECRETORY GRANULES IN THE CHROMAFFIN CELLS OF THE ADRENAL GLAND

I. BENEDECZKY

Department of Zoology, Attila József University, Szeged
(Received December 1, 1982)

Abstract

The author studied the granulogenesis of chromaffin cells in the adrenal gland of golden hamster at ultrastructural level. It was found that the Golgi apparatus of the glandular cells was in tight morphological relationship with the rough surfaced endoplasmic reticulum ,mitochondria and various types of vesicles. The development of the prosecretory granules was observed first of all in the terminal vesicles of the Golgi apparatus. A significant amount of degranulated tubules of the endoplasmic reticulum was also detected in the direct neighbourhood of the Golgi apparatus, where even the detachment of the smooth surfaced vesicles was observable on the terminal parts of the tubules. It was presumed that the smooth surfaced vesicles transport secretory proteins from the rough surfaced endoplasmic reticulum to the elements of the Golgi apparatus. Studying the granulogenetic role of the cell nucleus it was found that Actinomycin D and 5 Fluorouracil treatment significantly altered the chromaffin cells, especially the ultrastructure of the nucleus and the granulogenetic processes representing hormone resynthesis.

All these observations are in favour of the fact that the granulogenesis of the chromaffin cells stands under regulation even at cellular level; that is, this phase of the secretory process is nucleus-dependent.

Introduction

The biochemical analysis of isolated chromaffin granules has revealed that macromolecular material and substances of low molecular weight can be demonstrated in a rather significant amount in the granules (WINKLER, 1976). Amoung the macromolecular components proteins are present in the highest amount in the chromaffin granules, from which the chromogranin also contains amino sugars (SMITH and WINKLER, 1967) and sialic acid in 3% (BARTLETT and SMITH, 1974). Enzymes also occur among the protein components (dopamine β-hydroxylase, ATP-ase) and even the presence of cytochrome B 559 and certain flavoproteids could be demonstrated (FLATMARK et al. 1971). The high lizolecithin content of the chromaffin granules is striking (16.8% of the total amount of phospholipid), which may stand in connection with the exocytosis of the granules (WINKLER and SMITH, 1975).

The characteristic feature of the chromaffin granules is that the water soluble fraction contains a large number of material with low molecular weight, catecholamines and nucleotides (WINKLER and SMITH, 1975). In the knowledge of the mainer chemical components of the chromaffin argnules, arises the question; where and how many various chemical components develop and by which cell biological processes they "become packed" into the secretory granules? Certain events of the granulogenesis, like the incorporation of ^{3}H-labelled leucin into the chromogranin molecules, can be well followed by biochemical methods. Other partial processes are less clear for example the origin of the secretory granule membrane, the path and transport mechanism of the secretory proteins. The morphological relationship between the

Golgi apparatus and the endoplasmic reticulum in granulogenesis are not clear enough either, furthermore the cellular mechanism of the granulogenetic regulation and within this the direct morphogenetic regulatory role of the cell nucleus is almost completely unkpown.

Therefore, our studies aimed to describe the mode of formation of the chromaffin granules and to provide new data regarding the granulogenetic role of the cell nucleus on the basis of detailed ultrastructural analysis.

Materials and Methods

Golden hamsters *(Mesocricetus aureus)* used in our studies were obtained from the central animal house of the Oxford University. Before taking samples for electron microscopic studies the animals were narcotized with 3.5% chloral-hydrate, the chest was opened, cannula was led into the left ventricle of the heart, and the right auricle was incised with a single cut. Before perfusion fixation the circulatory system of the animals was rinsed with physiological salt solution, then after washing the blood perfusion fixation was carried out for 20—25 min. with a fixative mixture containing 2.5% glutaraldehyde (TAAB) and 4% formaline. Following perfusion the adrenal glands were taken out, cut into two pieces with a razor blade and further fixed in a refrigerator for 1 hr in the above fixative at 4 °C. Prefixation was followed by postfixation with osmium tetroxide in 2% OsO_4 lasting for 1—2 hrs, buffered according to Palade. Dehydration was carried out on ascending alcohol series and the samples were embedded in Durcupan ACM. The ultrathin sections were prepared with an LKB ultramicrotome. The sections were double contrasted, "stained" for 30 min. in saturated uranyl acetate diluted solution, and contrasted with lead citrate ofter being washed in destilled water.

Treatment of the experimental animals:

Before insulin treatment the animals were starved for 24 hrs with securing tap-water. A single i.p. treatment was carried out with insulin with the doses of 10 and 20 IU/100 g. 6 hrs following this, the animals received Actinomycin D (AD) and 5-Fluorouracil (5-FU) treatment. Doses of 250 ug/kg (AD) and 250 mg/kg (5-FU) were administered i. p.

Samples were taken for electron microscopic studies 3, 6, 24, 48, 72, 96, 120, 114 and 168 hrs after the insulin treatment. Following the insulin + AD and 5-FU treatment, respectively, the first samples were taken after 24 hrs, and the further time-points were the same. The electron microscopic pictures were prepared on a JEM 100 B type electron microscope.

Results

Morphological characteristics of granulogenesis in normal adrenal gland

Well developed Golgi area was observable in the direct neighbourhood of the cell nucleus in the chromaffin cells of the adrenal gland, as is general in glandular cells (Fig. 1).

The characteristic morphological feature of the Golgi area in the chromaffin cells was that many rough surfaced endoplasmic reticulum tubules and mitochondria could be found in loose substance, that is the membrane components forming the Golgi apparatus (saccules and vesicles) were in tight topographical connection with the afore mentioned cell organelles (Figs. 1, 2, 3).

The Golgi apparatus itself was mainly made up of saccular elements, 3—4 parallel saccules could be seen side by side (Figs. 1, 3). The Golgi vesicles occured in varying amount in the whole area of the Golgi, frequently in the direct neighbourhood of the saccules, but also further from them. Golgi vacuoles were only sporadically found in the chromaffin cells.

The size and morphological characteristics of the vesicles found in the Golgi area showed rather great variation. The smallest Golgi vesicles had diameters of

about 50 nm, the largest ones even reached 200 nm. The majority of the Golgi vesicles were electron lucent or moderately electron dense. Vesicles with spiny edges were also observable in significant number among the smooth surfaced Golgi vesicles (Figs. 2, 3, 4). The presence of electron dense material was frequently observed in the terminal vesicles of the Golgi saccules (Figs. 1, 2, 3, 4), but the prosecretory material was rarely present in the centrally developed vesicle of the saccule. The precursors of the secretory granules, the so-called prosecretory granules, were not only observable on the terminal part of the Golgi saccules, but also perisaccularly, in the whole Golgi area; in smaller-larger number (Figs. 1, 2, 3). The recognition and separation from the „mature" secretory granules was possible on the basis of their smaller size and the lower inner density.

Tight morphological relationship was detectable not only between the Golgi saccules and the prosecretory granules, but between the rough surfaced endoplasmic reticulum tubules and the newly formed granules as well. Thus, for example, the ribosomes were still well observable on the end of the membrane of the rough surfaced endoplasmic reticulum cistern which extended into the Golgi area (Fig. 3). However, the long tubule forming the extension of the cistern was already agranular and showed tight adjustment to the neighbouring secretory granules.

The development (Figs. 2, 3) and attachment of a few vesicles could also frequently be observed on the terminal part of the degranulated Er tubules, therefore it is probable that one part of the vesicles found in the Golgi area is of rEr origin.

Occasionally, the presence of so-called spiny sections was detectable on the central or terminal membrane of certain Golgi saccules. On the basis of the picture it cannot be decided whether these „spiny-edged" membrane sections were becoming attached or incorporated, nevertheless, their presence was noteworthy (Fig. 4), since the Golgi membranes are traditionally regarded to be smooth surfaced membranes. The rootlet of the centriole (Fig. 1), microtubules and multivesicular bodies were also observable in the Golgi area (Fig. 2).

The ultrastructure of the chromaffin cells of the adrenal gland following 5-Fluorouracil and Actinomycin D treatment

Following 5-Fluorouracil (5-FU) treatment so-called spotted nucleoli appeared in the majority of the nuclei of the chromaffin cells (Fig. 7). Due to their high electron density the „spots" were rather striking and were present in an even distribution in the substance of the nucleus. Both granular and filamentous components were detectable in the „spots" (Fig. 7). The 5-FU treatment rarely resulted in the development of ring-shaped nucleoli, too (Fig. 6).

Actinomycin D (AD) treatment produced the characteristic segregation of the nucleoli (Fig. 5). In the segregated nucleoli an electron dense granular and a fibrillar zone could be separated, and besides these the development of a lighter amorphous area was also detectable (Fig. 5). The cytoplasm was firstly characteristic of having lower amount of secretory granules, but the presence of many granules having irregular shape and low electron density was also a characteristic feature (Fig. 9).

The appearance of so-called quadrilamellar membranes in the chromaffin cells was also a new phenomenon (Fig. 8). They often developed in the direct neighbourhood of the cell nucleus, the inner membranes were agranular, and several ribosomes were observable on the outer ones. The Golgi apparatus was relatively small in the chromaffin cells treated with 5-FU (Fig. 9).

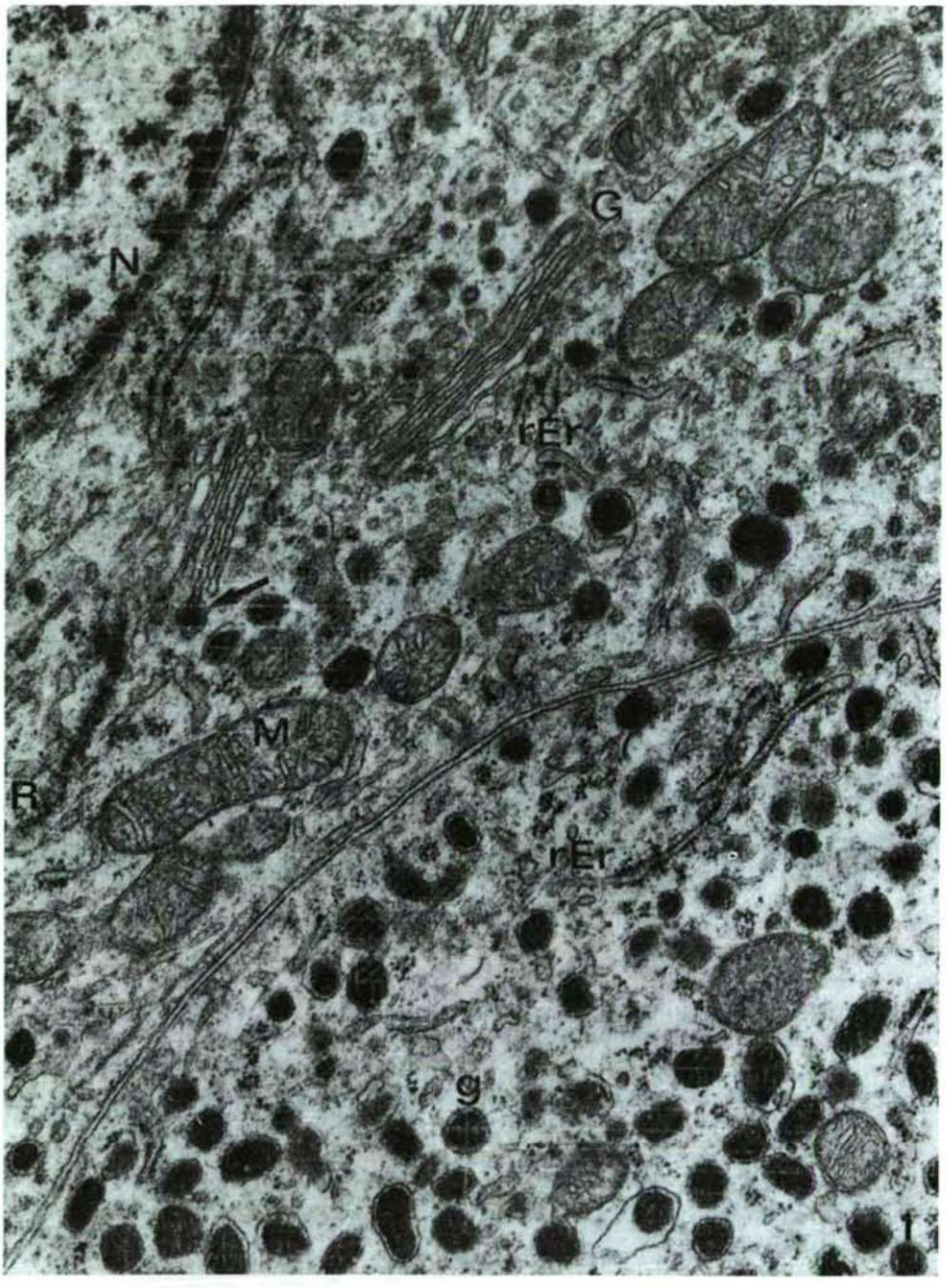

Fig. 1. Chromaffin cells of adrenal gland in untreated golden hamster. Extented Golgi area (G) can be seen beside the cell nucleus (N). The development of prosecretory granule is observable in the terminal cistern of some Golgi saccules (→). Several rough surfaced endoplasmic reticulum tubules (rEr) and mitochondria (M) can be seen in the Golgi area. R = rootlet, g = mature secretory granule. X 25 000.

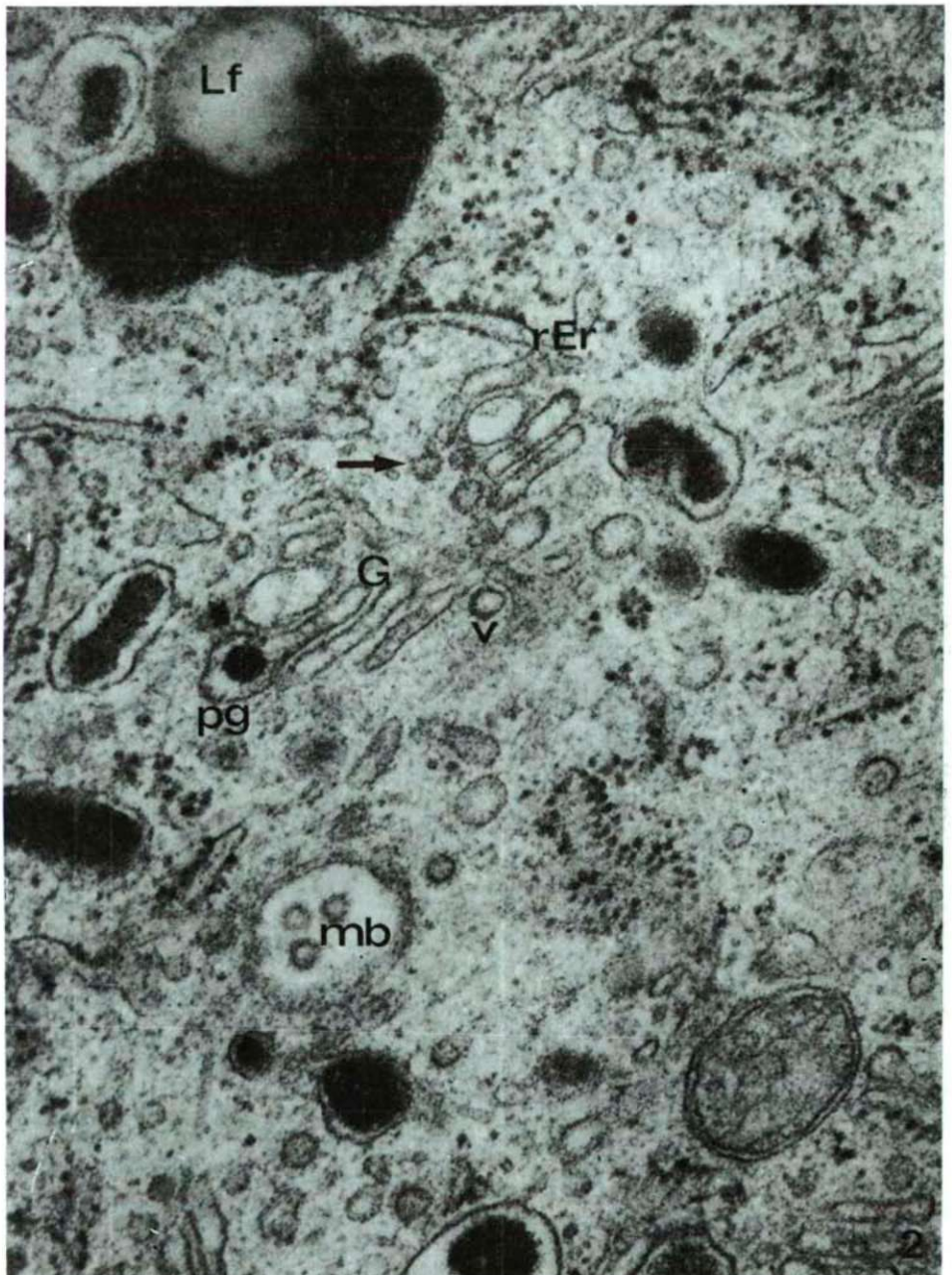

Fig. 2. Detail of Golgi apparatus (G). Several Golgi vesicules (v) are occupied beside the saccules. Degranulated tubules of rough surfaced endoplasmic reticulum (rEr) infolding into the Golgi area and a newly formed prosecretory granule (pg) are striking. The detachment of smooth surfaced vesicules from certain degranulated Er tubules is observable (→) Lf = lipofuscin granule, mb = multivesicular body. X 35 000.

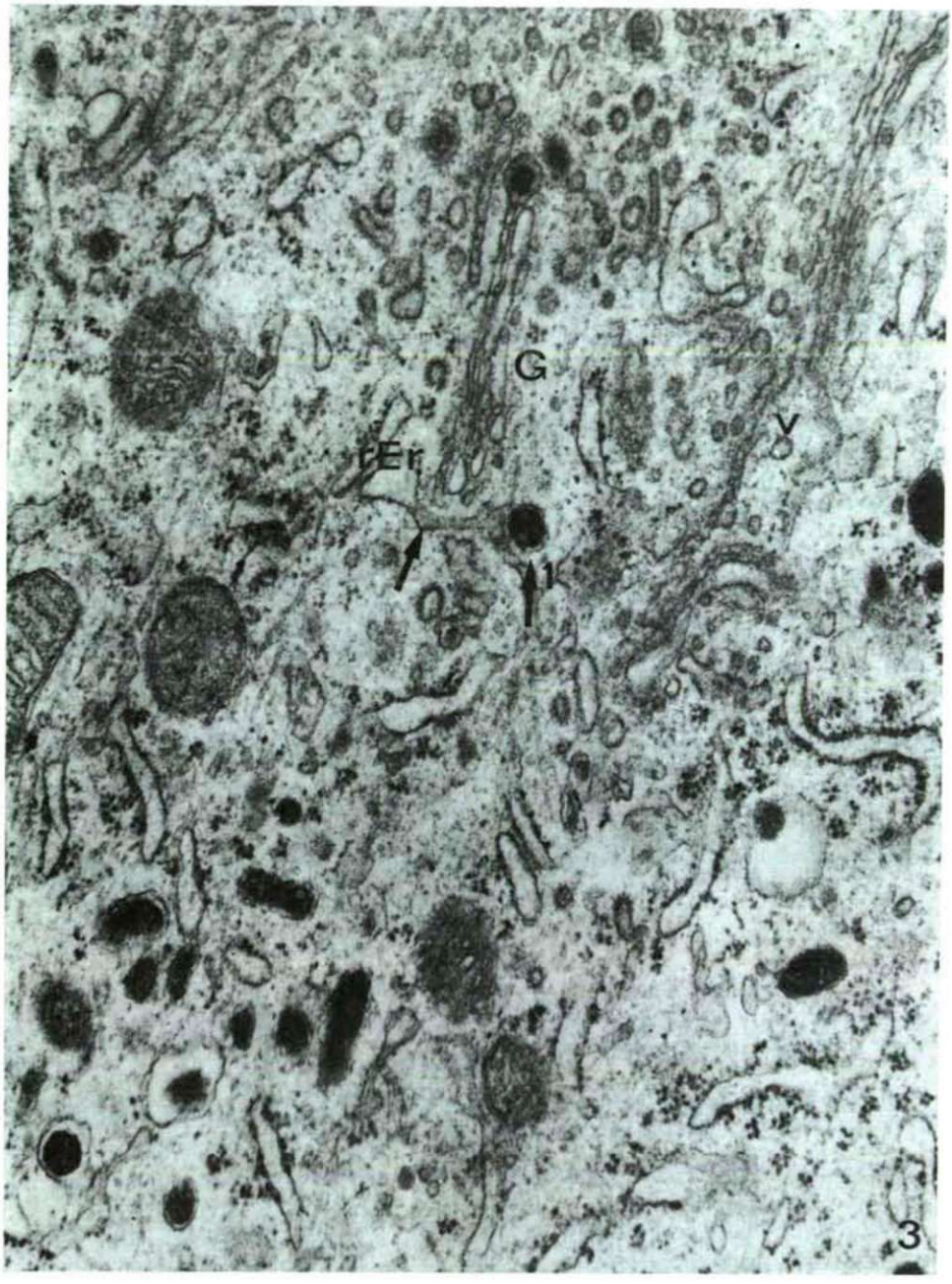

Fig. 3. Detail of chromaffin cell from untreated golden hamster adrenal gland. In the Golgi area (G) dilated rough surfaced endoplasmic reticulum tubules (rEr) are detectable in significant amount. The membrane surface of the tubules is partially degranulated (→). On some parts the tight morphological connection of the prosecretory granules and the degranulated endoplasmic reticulum is detectable (→ 1), v = Golgi vesicule. X 20 000.

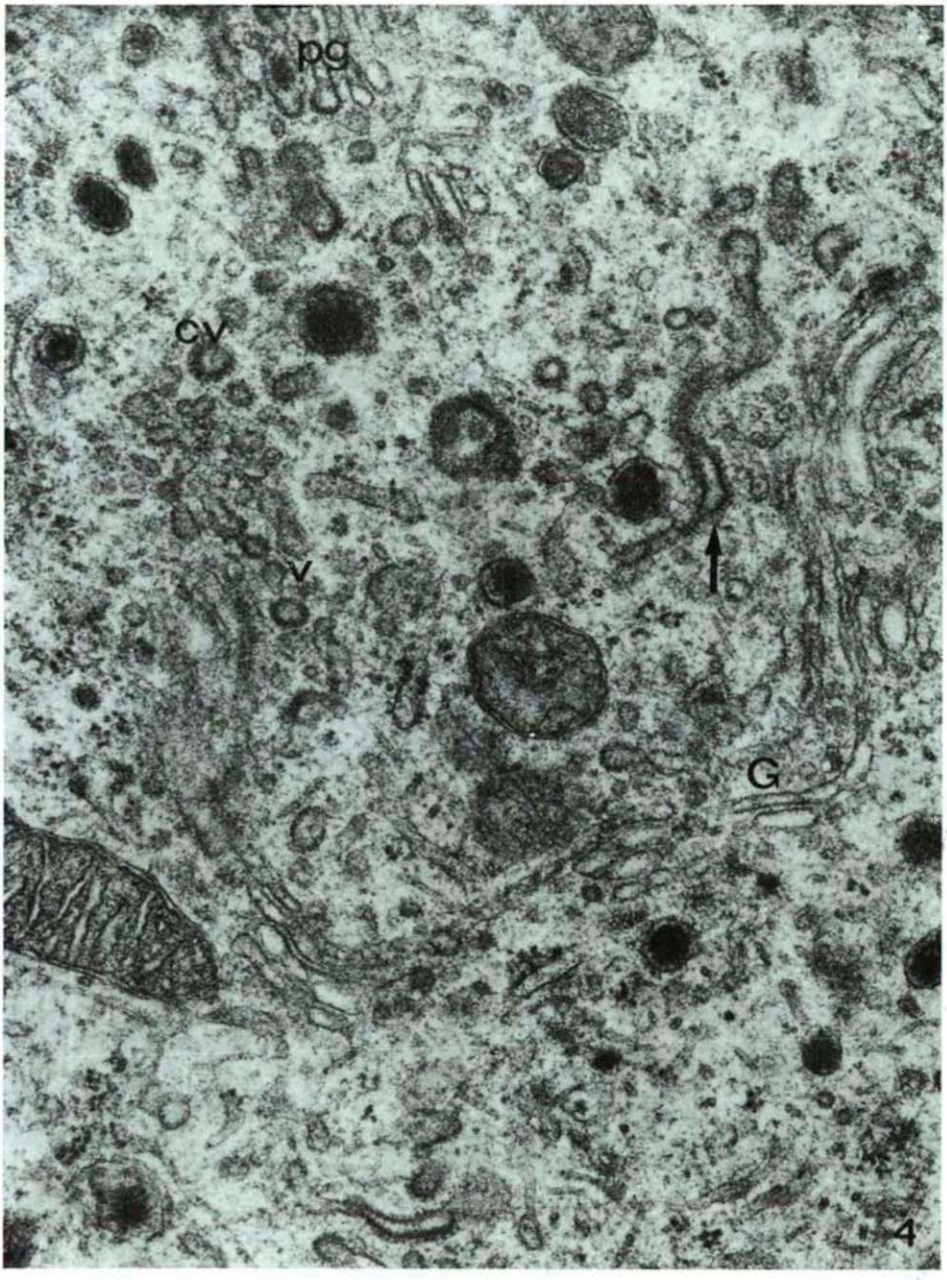

Fig. 4. High magnification of Golgi apparatus (G). The membrane of the Golgi saccule is covered by partial spiny coat (→). pg = prosecretory granule cv = coated vesicule, v = Golgi vesicule. X 62 000.

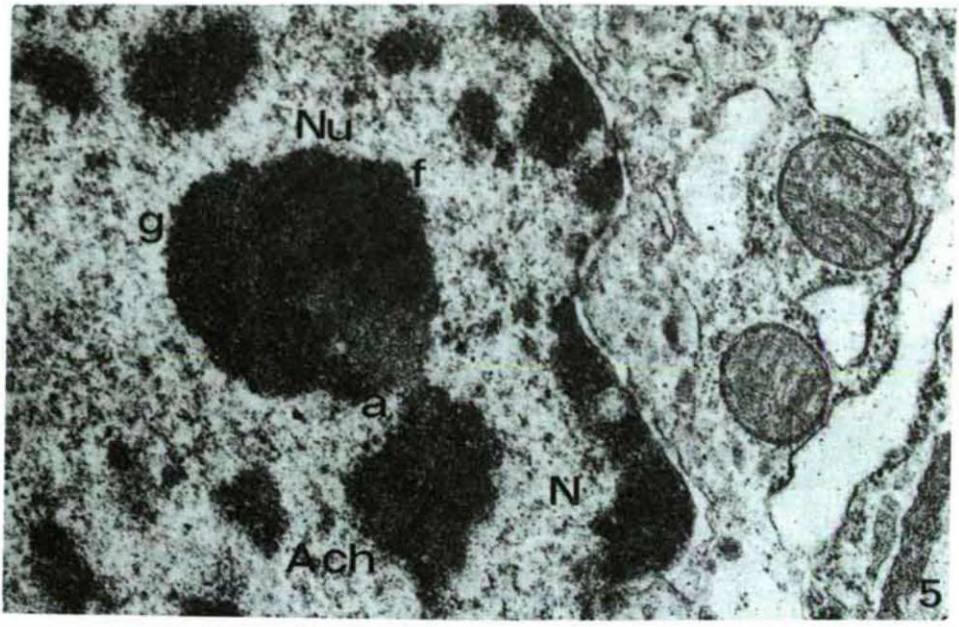

Fig. 5. Nucleus (N) of chromaffin cell 3 hrs following AD treatment. The substance of the nucleolus (Nu) is segregated to granular (g), fibrillar (f) and amorphous (a) components. Ach = associated chromatin. X 35 000.

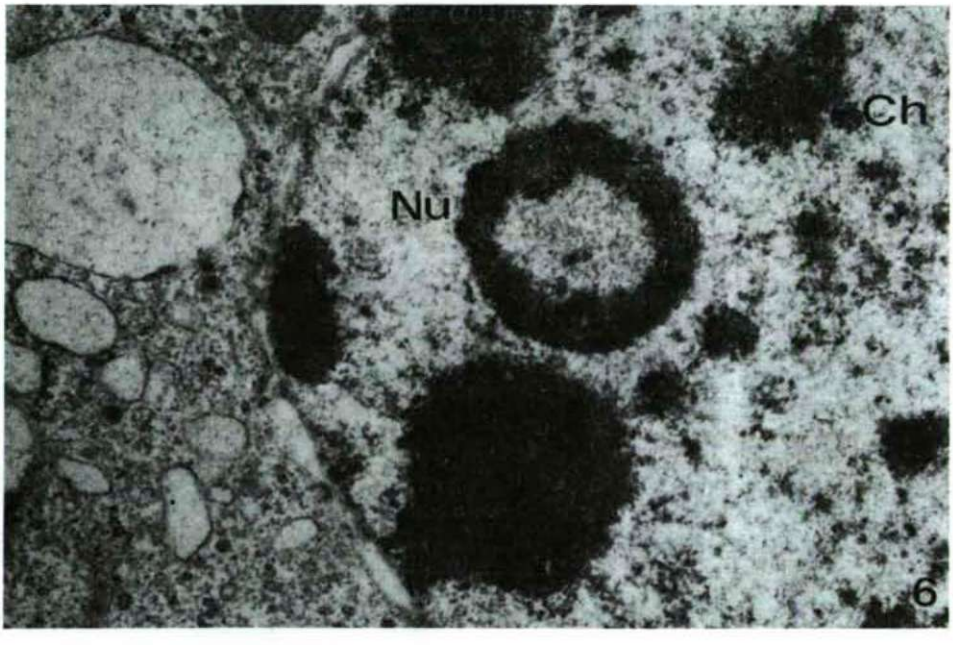

Fig. 6. Picture of "ringshaped" nucleolus (Nu), 3 hrs following 5-FU treatment. The phenomenon of segregation is partially observable. Ch = chromatin.

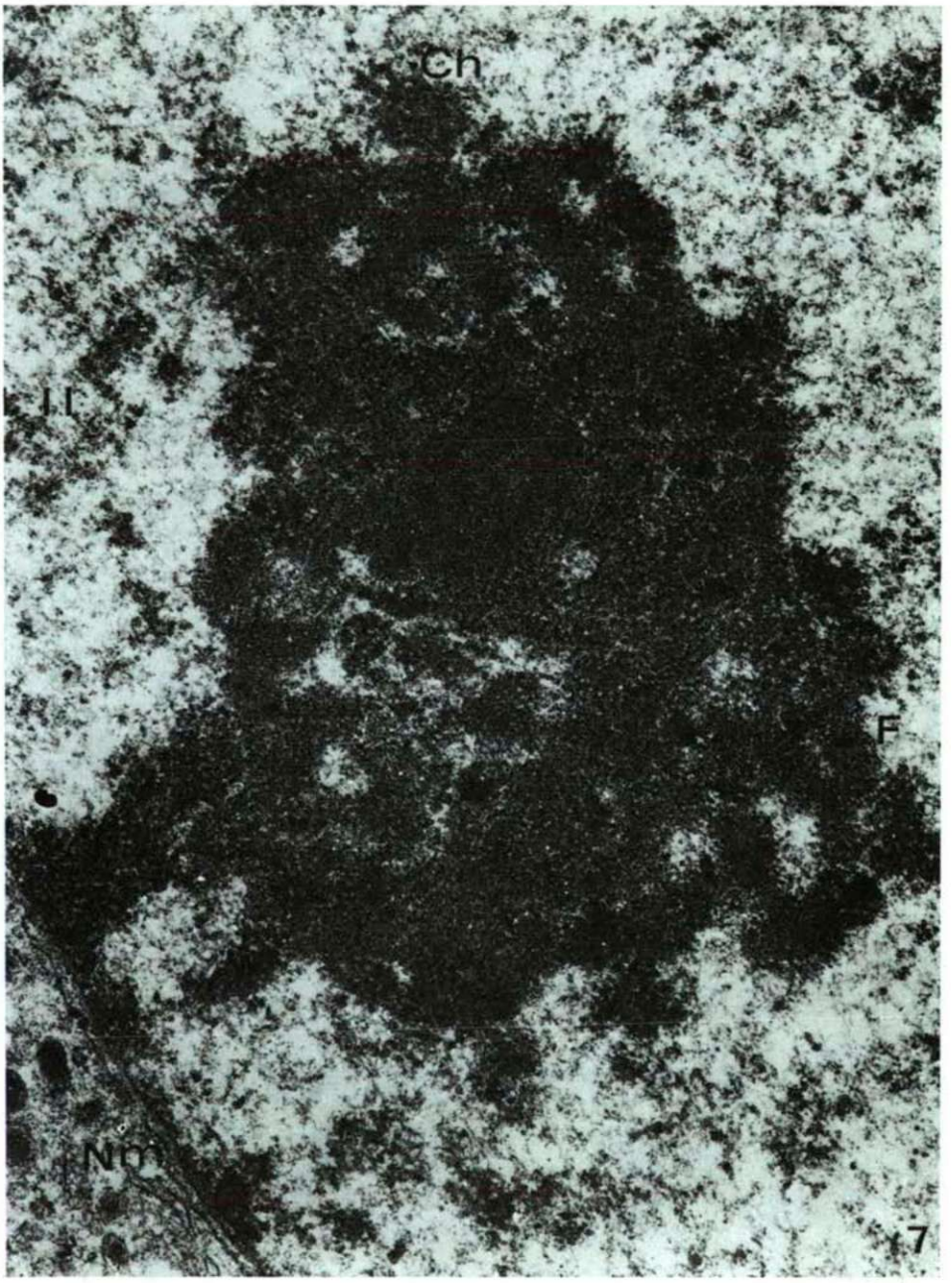

Fig. 7. "Spotted nucleolus" in the nucleus of chromaffin cell 72 hrs after treatment with 5-FU. Electron dense "spots" are striking in the nucleolonema having loose substance. F = fibrillar components, Nm = nuclear membrane, Ch = chromatin. X 75 000.

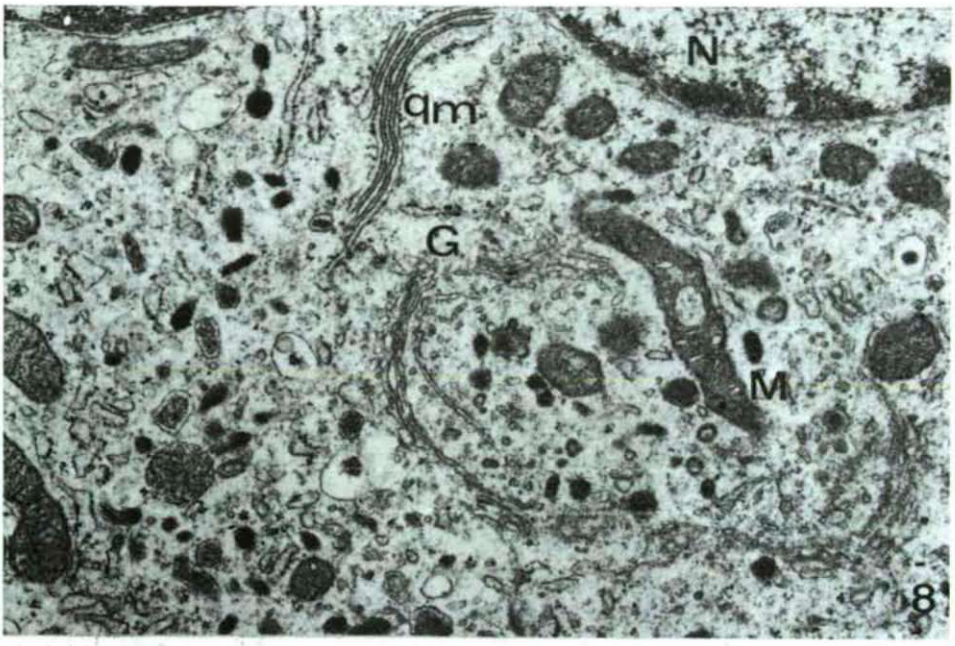

Fig. 8. Detail of chromaffin cell 48 hours following AD treatment. A quadrilamellar membrane (qm) can be observed in the neighbourhood of the cell nucleus (N), from the inner membrane surface of which the ribosomes are missing. The Golgi area (G) is relatively small. Certain mitochondria (M) are vacuolised. X 21 000.

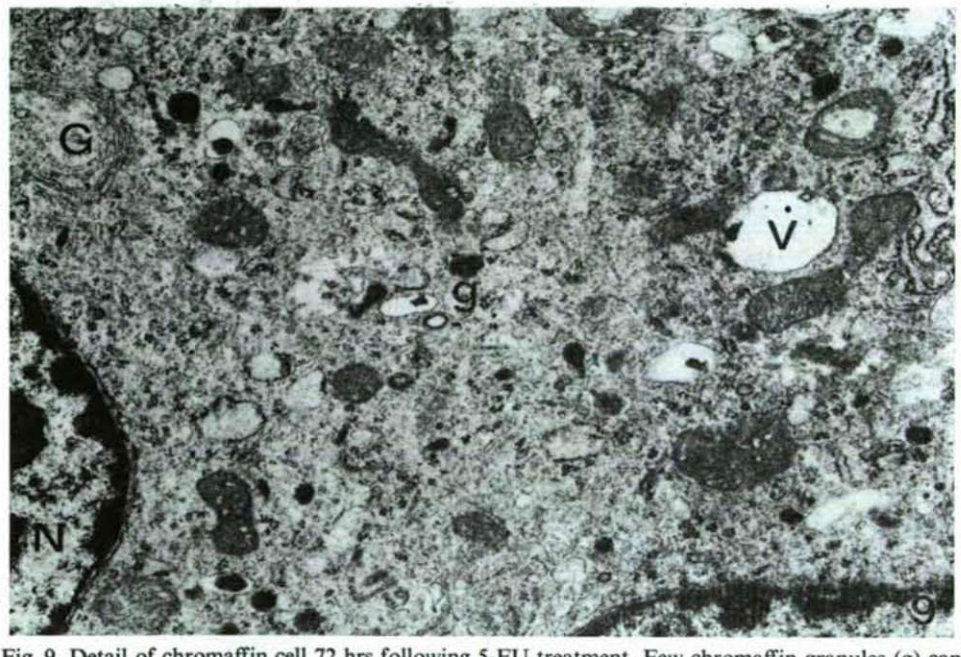

Fig. 9. Detail of chromaffin cell 72 hrs following 5-FU treatment. Few chromaffin granules (g) can be seen in the cytoplasm. The Golgi area (G) collapsed and does not contain prosecretory granule. Several empty vacuoles (V) are observable in the cytoplasm. N = nucleus, X 18 000.

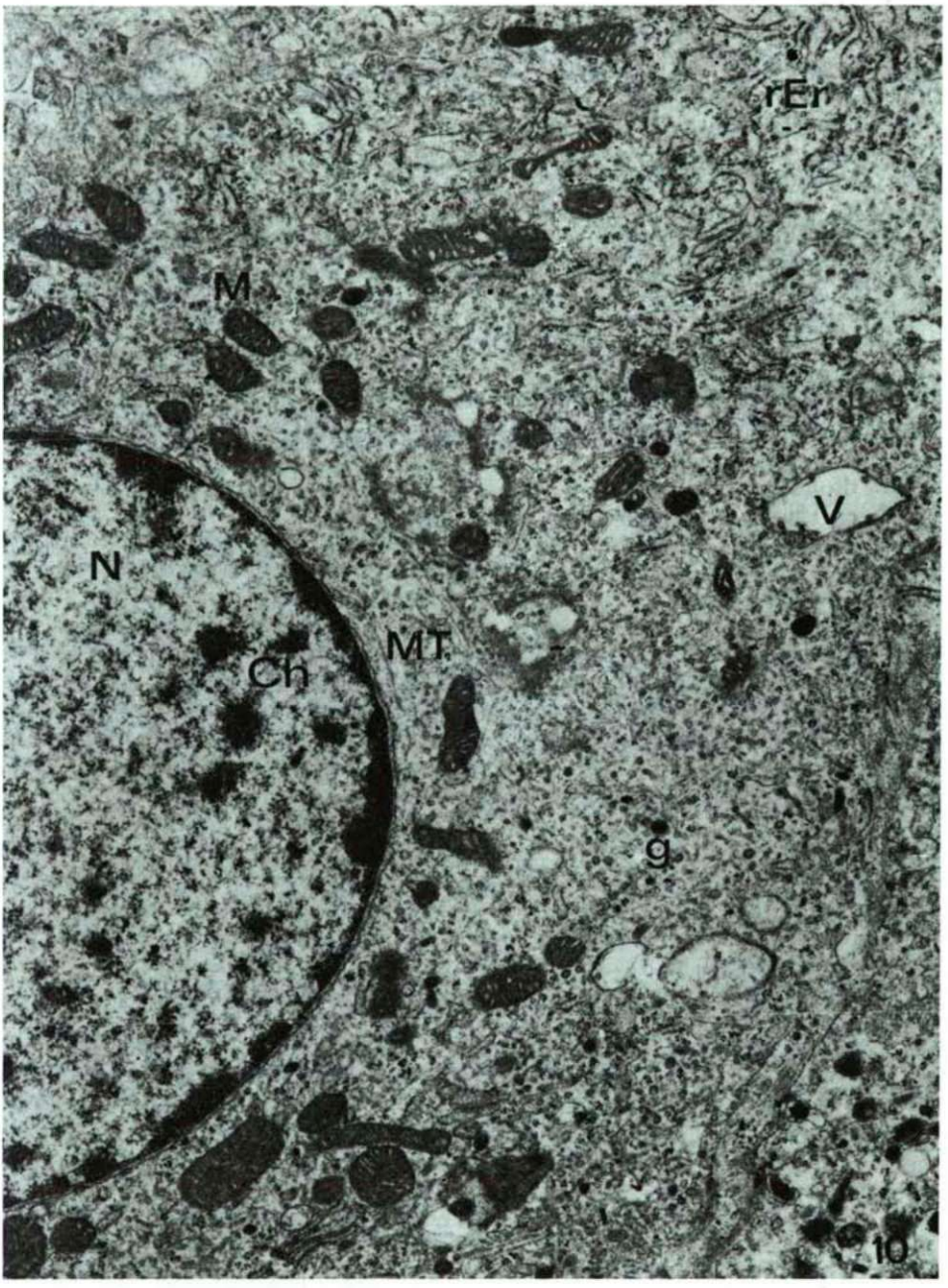

Fig. 10. Chromaffin cell 48 hrs after AD treatment. The structure of the cell nucleus (N) is well preserved, but it is rather poor in chromatin (Ch). In the cytoplasm many electron dense mitochondria (M), large number of rough endoplasmic reticulum tubules (rEr) and free ribosomes can be seen. Besides the cytoplasmic vacuoles (V) the small amount of secretory granules (g) is striking. MT = microtubule. X 15 000.

In certain mitochondria the appearance of vacuoles was also detectable following 5-FU treatment (Fig. 9). Ribosome aggregates, rough surfaced endoplasmic reticulum tubules were observable in large amount in the cells treated with 5-FU and AD (Figs. 9, 10).

Although the structural preservation of the chromaffin cells was rather good following 5-FU and AD treatment, and furthermore, abundant rEr and mitochondria were present in the cytoplasm — the development of new secretory granules was hardly detectable in the chromaffin cells; referring to the fact that the process of granulogenesis significantly altered due to the treatment with the above agents.

Discussion

Studies had been started rather long ago on the genesis of the secretory granules of the chromaffin cells in the adrenal gland (LEVER, 1955; WETZSTEIN, 1957; FUJITA et al. 1965; BENEDECZKY, 1966, 1969; RATZENHOFER and MÜLLER, 1967; ELFVIN 1967).

On the basis of these studies a conception developed that the granulogenetic process of the chromaffin cells of the adrenal gland is rather similar to that of described in other glandular cells (SIEKEWITZ and PALADE, 1958; FARQUHAR and WELLINGS, 1957; PALADE et al. 1962; SJÖSTRAND, 1962; CARO and PALADE, 1964; MUNGER, 1964; HERMAN et al. 1964; DASS and BAYLEY, 1965; REDMAN et al. 1966). Regarding the formation of the chromaffin granules HOLTZMAN and DOMINITZ (1968) set forth a new conception. During the course of their cytochemical studies they observed the presence of acid phosphatase in the secretory granules, therefore they proposed the Gerl-origin of the secretory granules. However, regarding the acid phosphatase content of the chromaffin granules BENEDECZKY and SMITH (1972) propounded that it may originate from the fusion of the coated vesicles and the prosecretory granules, during the course of which the coated vesicles, as primary lysosomes, contain and transport the enzyme in question to the secretory granules. Furthermore, BENEDECZKY and SMITH (1972) also proposed that the coated vesicles may transport other secretory base materials, too. Thus, for example, they may take part in the transport of the secretory proteins from the rEr towards the Golgi saccules; as also reported by JAMIESON and PALADE (1967), in the case of pancreas. The coated vesicles may transport proteins and parts of the membrane from the Golgi cisternae towards the prosecretory granules (BENEDECZKY and SMITH, 1972), and bypassing the Golgi apparatus, they may transport secretory proteins from the rough surfaced endoplasmic reticulum directly to the prosecretory granules.

All these presumptions prove that even in the begining of the 70s several basic processes were unclear in the mechanism of formation of the chromaffin granules. As cytomorphology and cytochemistry enriched our knowledge on the Golgi area with newer data, the picture regarding the genesis of the chromaffin granules became more and more complex. Firstly it became certain that the large number of proteins present in the chromaffin granules —; the synthesis of the chromogranin begins in the rough surfaced endoplasmic reticulum (WINKLER et al. 1972; BAUMGARTNER et al. 1974; GEISSLER et al. 1977). Concerning how the chromogranin reach up to the prosecretory granules, COUPLAND and KOBAYASHV (1976) provided new data by their autoradiographic studies. However, it is a principle problem in the work of COUPLAND and KOBAYASHY (1976) that they were unable to detect the accumulation of ^{3}H leucin in the rough surfaced endoplasmic reticulum before the incorporation into the Golgi apparatus. To surmount this problem authors declared that the chromaffin granules are poor in rEr. This statement cannot be strengthened on the basis of our earlier

studies (BENEDECZKY and SMITH, 1972) as well as our present results, and we unambiguously found that the chromaffin cells are rich in rEr elements.

During our observations we also emphasized that the rEr tubules are present exactly in the Golgi area — impacted among the saccules, being in tight topographical relationship with them; therefore we interpret the autoradiographic studies of COUPLAND and KOBAYASHY (1976) as follows: The incorporation of ^{3}H leucin observed at the early time-point (15 minutes) begins in the rEr tubules found in the Golgi area and only reaches as far as the Golgi saccules later (if doing so at all!). Now we have reached the critical question of granulogenesis; how the secretory proteins get from the rough surfaced endoplasmic reticulum to the Golgi saccules, where according to the classical conception the final encasement takes place.

According to JAMIESON and PALADE (1967) the secretory proteins are transported to the elements of the Golgi apparatus through the so-called transitional vesicles, becoming unattached from the rEr. Since we frequently observed the detachment of degranulated vesicles on one end of the rEr tubules found in the Golgi area, in agreement with the opinion of JAMIESON and PALADE (1967) we also think that the secretory proteins reach the saccules of the Golgi apparatus through these vesicles — where the uptake of the rest of the secretory materials also takes place (sugars, sulphate groups, etc.).

The uptake of the other components of low molecular weight of the chromaffin granules, like the nucleotides and hormones, is well clarified on the basis of biochemical and pharmacological studies (WINKLER, 1977; ABERER et al. 1978) and is not so followable by ultrastructural methods. The role of the cell nucleus is a point of further interest in the process of secretion. It is known on the base of literary data (LEEMAN, 1959a, b; ROELS, 1963; VIOLA MAGNI, 1968) that the cell nucleus shows various alterations in the secretory phases produced in different ways. The nature of these changes, however, is not always unambiguous, thus, not even the conclusions which could be drawn. In our studies we established such a secretory model where first the hormone content of the chromaffin cells was "depleted" by insulin loading (BENEDECZKY et al. 1965, BENEDECZKY, 1967), then following this 5-FU and AD treatment was carried out and the process of hormone resynthesis was studied in the function of the altered nucleic acid metabolism (BENEDECZKY et al. 1972). It was determined that both agents inhibited the process of hormone resynthesis strongly and durably (BENEDECZKY et al. 1972). It is known that AD it preferentially binds to chromatin in the early time-points following treatment, hampering in such a way the transcription of RNA by the inhibition of the RNA polymerase (HURVITZ et al. 1962; GOLDBERG and RABINOVITZ, 1962; REICH, 1964; RINGERTZ et al. 1969; RECHER et al. 1971). As a consequence of the inhibition of the process of transcription a long-standing disturbance takes place in the RNA metabolism (GIRARD et al. 1964; PENMAN, 1966; WEINBERG etal. 1967; SINGER and PENMAN, 1972; COPOER and BRAVERMAN, 1977). 5-FU, besides its direct DNA synthesis inhibitory effect (HEIDELBERGER, 1963), also influences the metabolism of RNA; it infiltrates into the RNA, occupying the place of uracil (KOPPER et al. 1972).

Both 5-FU and AD bring forth characteristic ultrastructural alterations in the cell nuclei (LAPIS and BENEDECZKY, 1966; STENRAM, 1969). These characteristic alterrations — spotted nucleolus, ring-shaped nucleolus, nucleolus segregation — also developed in the nucleus of the glandular cells; therefore we came to the conclusion that the applied agents significantly changed the nucleic acid metabolism of the glandular cells.

The consequences of the altered nucleic acid metabolism were also detectable in

the cytoplasm. We have proved by our biochemical measurements (BENEDECZKY et al. 1972) the inhibition of hormone resynthesis, and simultaneously, we observed the large degree of decrease in the number of the hormone-storing granules in the cytoplasm of the chromaffin cells. Many irregular shaped granules of low density were present among the granules found in the cytoplasm, which may also be brought into connection with the previous 5-FU and AD treatment, respectively. The appearance of quadrilamellar membranes, furthermore, the development of small "collapsed" Golgi apparatus may be evaluated as degenerative ultrastructural signs. However, besides the degenerative ultrastructural changes caused by the agents, regenerative signs were also detectable. The increase in the amount of free ribosomes, polyribosomes and the Er tubules (mainly smooth Er) in the cytoplasm of the chromaffin cells was especially striking. All these ultrastructural signs refer to the fact that through "feedback mechanism" the hormone release and depletion-developing on the effect of the insulin treatment-set forth the resynthetic activity of the medullary cells. It is known, for example, that the amount of dopamine β-hydroxylase and tyrosine hydroxylase increases significantly in the adrenal gland following intensive hormone depletion (VIVEROS et al. 1969; THOENEN, 1975). The synthesis of these enzymes proably stands in tight connection with the rough surfaced endoplasmic reticulum of the glandular cells, and so it is understandable that this process resulted in the increase in the amount of rough surfaced endoplasmic reticulum. Namely, the inhibitory effect of the 5-FU and AD treatment applied under the resynthesis did not prevail immediately and some partial processes of the hormone resynthesis could come into action from the RNA reserves synthetised before the inhibition, however, this reserve was not enough for the complete process of granulogenesis, therefore the glandular cells became blocked in an initiative „rEr increased" state.

These data are in favour of the fact that the process of granulogenesis takes place only in glandular cells with intact nucleic acid metabolism, that is, the hormone resynthesis and process of granulogenesis are nucleus-dependent cell physiological processes.

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Address of the author:
PROF. DR. I. BENEDECZKY
Department of Zoology, A. J. University
H-6701 Szeged, P.O. Box 659.
Hungary

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HABITAT SPECIALIZATION OF LEAFHOPPER COMMUNITY LIVING IN A SANDY SOIL GRASSLAND

Gy. GYÖRFFY and T. POLLÁK

Department of Zoology, Attila József University, Szeged
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Abstract

Dispersion according to the macrohabitat of a leafhopper community formed by a population of 54 species, living on a mosaic-complex-like sandy soil grassland was examined using cluster analysis. 5 groups can be separated on the ground of habitat-specialisation degree. 39% of species can be found in wind-furrows with more favourable environmental effects and 15% only on dry sand-hills. Frequency of 18% is the same in both habitats, 15% was more frequent in wind-furrows, 13% on sand-hills. Species groups can be reduced on a higher and higher similarity level, with a rising specialization degree in the direction of wind-furrows.
Key words: Leafhopper community, habitat specialization, grassland, cluster analysis.

Introduction

Beside the quantitative analysis of leafhopper communities their study according to dominance relations, bionomics and ecological valency has also been done (GYÖRFFY, 1980a, 1982) in the cource of complex ecological studies of the territory of Kiskunság National Park (Hungary) since 1976 (MÓCZÁR et al., 1980). The leafhopper populations living on the two terrains of the model area are sharply different both in species composition and in dominance as it appears from the above mentioned data. As mosaic character and concomitant local biotopdifferences can make the animal communities of an ecosystem rich and so they can influence their stability on a large scale (BANACH et al., 1979; and others), it is reasonable to look for these populations which can take most part in the functional connections of mosaics, respectively for those ones which become localised only on one of the habitats. Another advantage of stating habitatpreferency is that the importance of habitat dimension in realization the coexistence of leafhopper populations can be cleared up. SCHOENER (1974) stated that habitat can be many times more important than food-type. MÜLLER (1980) found that spatial dispersion of leafhopper communities is determined by host-plant relations not so much as by the animals' demands on microclimate. DENNO (1980) puts food-type-dimension (microhabitat) on the first place though he devided height above surface between 0—25 cm only, while there some meters differences in height at Müller's plant associations (1980). Our investigation -area is similar to the latter one, so division of habitat dimension is doubtless reasonable.

Investigated area and the methods

The investigated area in the Kiskunság National Park (Hungary) has a mosaic-complex character caused by the occurence of sand-hills and wind-furrows. These two terrains are different not only in their height but — depending on it — in their microclimate, plant associations, too (KÖRMÖCZI et al., 1981). On the highest places of sand hills *Festucetum vaginatae danubiale normale*, respectively degraded form of *Potentillo-Festucetum pseudovinae danubiale euphorbietosum sequerianae* and its

Bromus tectorum facies exist. Wind-furrows, laying 1.5—2 m deeper are covered partly by *Festuca pseudovina* facies of *Lolio-Potentilletum anserinae*, the deepest parts are occupied by *Festuca pseudovina* facies of *Molinio-Salicetum rosmarinifoliae* (BODROGKÖZY and FARKAS, 1981).

Sampling were carried out from March to November, 1977 to 1981; between 1977 and 1979 monthly, in 1980—81 every second week. "Suction trap" was used (GYÖRFFY, 1980b) collecting 10—10 samples on both terrains. The collected material was selected by hand from debris in the first time, later, from 1978 by the xylene-method of MARTSON and HENNESSEY (1978).

For stating habitat preference the yearly average individual days were determined for both terrains converting the basic data into pieces/m² units. The distribution of the resulted occurence frequency data between the two terrains was taken as an index of habitat-preferency. From the average and standard deviation of five years' data we stated the certainty of habitat-preference of the single populations with the help of t-test choosing 5, resp. 10% as significancy level. Similarity level of dispersion of the single populations by habitat was determined by Renkonen index. We tried to separate resp. to choose species groups behaving alike by cluster analysis done with the method of weighted average using similarity matrix, which was constructed on the basis of Renkonen values.

Results and discussion

54 species — of the 94 ones known on area up to this time (GYÖRFFY, 1982) — remained in the course of the exact quantitative examinations the poulations of which gave enough data for the present study. These are found in Table 1., in alphabetical order, where we can find beside serial number (later we mark the single populations with their serial number) the rate of dispersion by terrains resp. and wether it is significant or not.

Representing the dispersion data of Table 1. on a diagram in the order of their size we get a more exact picture of dispersion by terrains for the whole leafhopper community (Fig. 1). It can be seen that significancy is higher at the end values, 17 species occur significantly (on a level of 5%) in wind-furrows, 5 ones on sand-hills. Besides these the dispersion rate of 8 species is significant on the same level. If we choose a significancy level of 10%, 4 further populations join the group of wind-furrow-friends, while the number preferring sand-hill rises with 3 populations. That is, outer groups stick stronger to the occupied biotop, while the intergrade populations are moving freely between the two habitats.

Table 1. Percentile distribution of the most frequent populations between the two terrains (s.n.: serial number, m. d. p. c.: mean distributional per cent, l. s.: level of significancy, wf: wind-furrows, sh: sand-hills)

s. n.	Species	m. d. p. c. wf	m. d. p. c. sh	l. s.
1.	*Anaceratagallia ribauti* OSS.	73.99	26.01	<0.05
2.	*Anakelisia perspicillata* BOH.	99.10	0.90	<0.05
3.	*Aphrodes albiger* GERM.	93.34	6.66	<0.05
4.	*Aphrodes bicinctus* SCH.	91.17	8.83	<0.05
5.	*Aphrodes elongatus* LETH.	40.14	59.86	n. s.
6.	*Arboridia parvula* BOH.	26.37	73.63	n. s.
7.	*Artianus interstitialis* GERM.	14.44	85.56	<0.05
8.	*Austroagallia sinuata* M. R.	46.51	53.49	n. s.
9.	*Batracomorphus irroratus* LEW.	57.61	42.39	n. s.
10.	*Bobacella corvina* HORV.	95.25	4.75	<0.05
11.	*Dictyophara pannonica* GERM.	0.00	100.0	<0.05
12.	*Delphacodes albifrons* FIEB.	100.00	0.00	<0.05
13.	*Deltocephalus pulicaris* FALL.	99.47	0.53	<0.05
14.	*Doratura homophyla* FLOR.	16.94	83.06	n. s.
15.	*Doratura stylata* BOH.	77.85	22.15	<0.05
16.	*Eupelix cuspidata* F.	50.00	50.00	n. s.
17.	*Eupteryx notata* CURT.	96.09	3.91	<0.05
18.	*Eurysula lurida* FIEB.	99.55	0.45	<0.05

19.	*Euscelis incisus* KIRSCHB.	59.36	40.64	n. s.
20.	*Goniagnathus brevis* H. S.	79.43	20.57	<0.1
21.	*Graphocraerus ventralis* FALL.	100.00	0.00	<0.05
22.	*Gravesteiniella boldi* SCOTT.	99.09	0.91	<0.05
23.	*Hecalus glaucescens* FIEB.	5.06	94.94	<0.05
24.	*Jassargus sursumflexus* THEN.	100.00	0.00	<0.05
25.	*Jassidaeus lugubris* SIGN.	72.95	27.15	n. s.
26.	*Kelisia brucki* FIEB.	100.00	0.00	<0.05
27.	*Kelisia perrieri* RIB.	80.00	20.00	n. s.
28.	*Kelisia vittipennis* SAHLB.	100.00	0.00	<0.05
29.	*Kosswigianella exiqua* BOH.	16.04	83.96	<0.1
30.	*Kosswigianella spinosa* FIEB.	100.00	0.00	<0.05
31.	*Lepyronia coleoptrata* L.	57.09	42.91	n. s.
32.	*Macustus grisescens* ZETT.	93.90	6.10	<0.1
33.	*Megophthalmus scanicus* FALL.	100.00	0.00	<0.05
34.	*Mendrausus pauxillus* FIEB.	17.35	82.65	<0.05
35.	*Metadelphax minuscula* WAGN.	33.33	66.67	n. s.
36.	*Mocydiopsis parvicauda* RIB.	85.71	14.29	<0.1
37.	*Neoaliturus fenestratus* H. S.	66.14	33.86	n. s.
38.	*Neophilaenus campestris* THUNB.	29.08	70.92	n. s.
39.	*Neophilaenus lineatus* L.	95.18	4.82	<0.05
40.	*Ommatidiotus inconspicuus* STAL.	22.61	77.39	<0.1
41.	*Paluda preyssleri* H. S.	85.44	14.56	<0.05
42.	*Paluda vitripennis* FLOR.	33.31	66.69	n. s.
43.	*Philaenus spumarius* L.	64.92	35.08	n. s.
44.	*Psammotettix alienus* DHLB.	10.14	89.86	<0.05
45.	*Psammotettix confinis* DHLB.	69.38	30.62	n. s.
46.	*Psammotettix provincialis* RIB.	9.65	90.35	<0.05
47.	*Psammotettix slovacus* DLAB.	100.00	0.00	<0.05
48.	*Recilia schmidtgeni* WAGN.	22.46	77.54	<0.1
49.	*Ribautodelphax albostriatus* FIEB.	96.81	3.19	<0.05
50.	*Tettigometra impressopunctata* DUF.	100.00	0.00	<0.05
51.	*Trypetimorpha fenestrata* A. COSTA.	90.56	9.44	<0.05
52.	*Turrutus socialis* FL.	79.14	20.86	<0.05
53.	*Ulopa trivia* GERM.	65.01	34.99	n. s.
54.	*Zyginidia pullula* BOH.	52.53	47.47	n. s.

According to fig. 1 this intergrade group consists the populations of the most species, that is they play an important part in the leafhopper community of both terrains. If we want to segregate specialist and generalist species according to their biotop, we have to determine the rate of dispersion which means a limit—value. This can be 5:95%, when 17 spp are specific for wind-furrows, 2 for sand-hills and 35 ones are generalists; or 10:90% can be choosen, when 21 species are specific for wind-furrows, 4 for sand-hills and 29 are generalists. But this choice is rather arbitrary. To get more objective picture about grouping of populations by habitats we used a cluster analysis. Fig. 2 shows a cluster obtained as a result of an analysis made on the ground of the similarity matrix of dispersion rates — it's not published owing to lack of space. The order of species corresponds to that of fig. 1. It can be seen from the first approximation that it is more correct to distinguish 5 spp groups instead of the original 3 ones. The greatest of them is the group specific for wind-furrows, which consists 21 spp, 39% of the total species (12—51 groups in the cluster). Only 8 species (34—11) are specific for the sand-hills having extrem environmental effects, that is, 15% of the total species number. 10 spp (18%) can be taken for typical generalists, with serial number 45—8. To every specialist group joints one intergrade one. Populations, marked with numbers 36—25 mean wind-furrow-friend (8 spp = 15%), 5—48 ones (7 spp = 13%) sand-hill-friend intergrade groups.

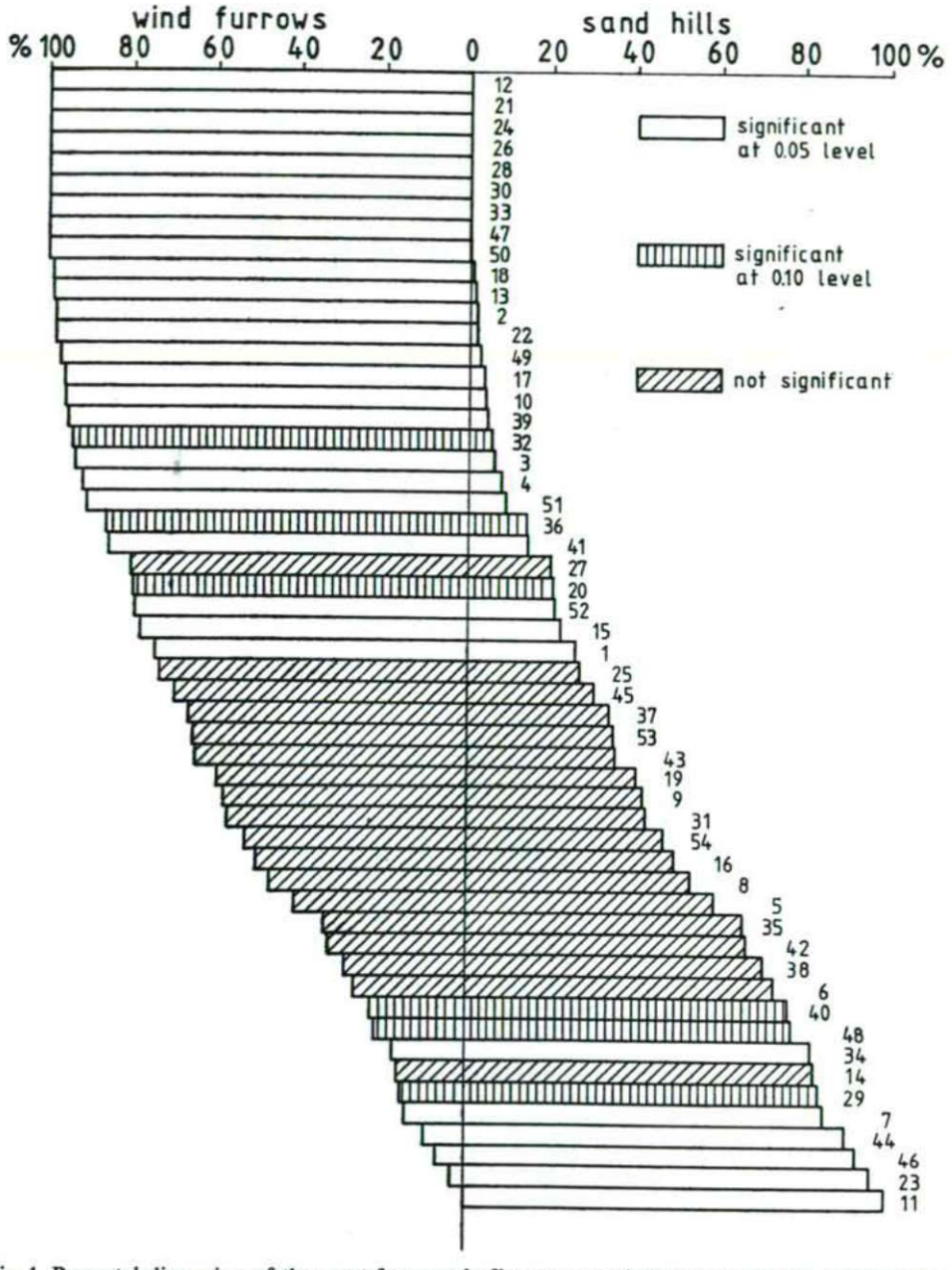

Fig. 1. Percental dispersion of the most frequent leafhopper populations between the two terrains (s. l. = level of significancy; numbers mean the serial numbers of species, see table 1.)

If we take into consideration reducing levels, as well, can be stated that these levels are rising with the rise of wind-furrow specifity, that is, the groups are getting more and more uniform. The determining character of wind-furrows having favour-

able environmental effects is proved by the joining groups on a higher and higher reducing level in the direction of wind-furrows (0.36, 0.66, 0.82).

Summarizing, it can be stated that in a habitat with mosaic-complex character the mosaic parts with more favourable environmental effects have a determining role in the intense habitat specialisation of leafhopper communities even if — as in our case — their rate is lower. A greater part of species sticks to this habitat (39%), while 15% is absolutely missing. If we want to study the functional connections between leafhop-

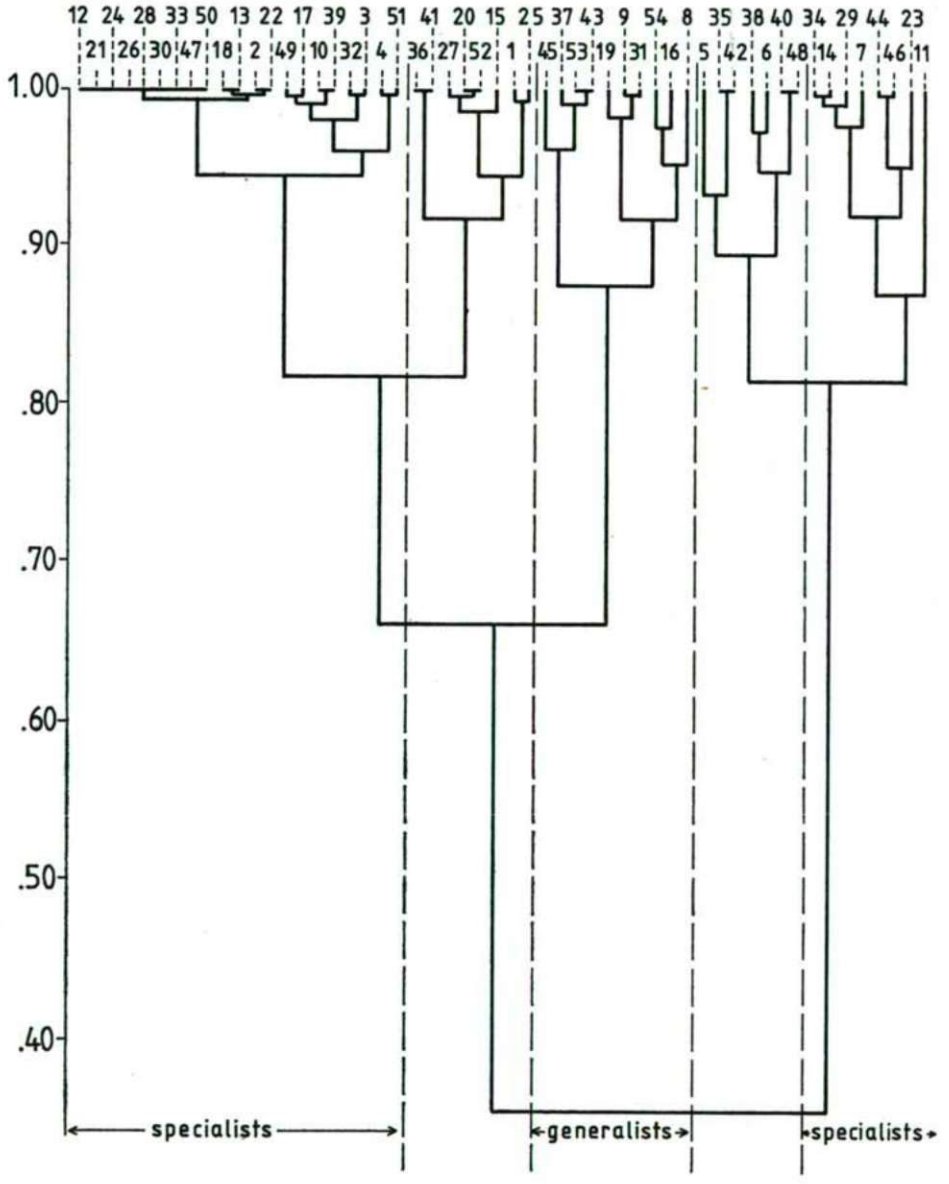

Fig. 2. Dendrogram of dispersion by habitats (serial numbers mean the same spp as in table 1.)

per populations of the two terrains, we can see that 10 spp (18%) take part most intensively in that, the populations of further 15 spp have to be taken into account as well. If the rate of dispersion between habitats is a result of a competetive situation, it can be used for examining the efficiency of it. The decision of this fact needs further examinations.

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Address of the authors:
DR. GY. GYÖRFFY
T. POLLÁK
Department of Zoology,
A. J. University, H-6722 Szeged,
P. O. Box 659, Hungary

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THE FLUORIDE CONTENT OF DRINKING WATER AND THE MENARCHEAL AGE

GY. FARKAS, A. FAZEKAS and ERZSÉBET SZEKERES

Department of Anthropology, Attila József University,
Clinic of Dental and Oral Surgery, Medical University of Szeged,
Public Health and Epidemiology Station of County Csongrád, Szeged
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Abstract

The mass prevention of caries by fluoridation method can only be done with adequate safety. For this it also has to be known whether in a given geographical or economic environment the regular and long-standing consumption of drinking water containing a fluoride-concentration optimal in regard of caries prevention influences destructively the normal physiological processes of the organism or not.

In this concern, authors studied the development and growth of school-aged children from two Hungarian settlements. The drinking water contains 1.09 mg/l of fluoride averagely in one of the settlements and 0.17 mg/l averagely in the other.

In the two, otherwise rather similar settlements (Kunszentmárton and Kiskunmajsa) no main variations were found in the puberty age (menarcheal age) of the schoolgirls. This supports the presumption of authors according to which the intake of fluoride optimal in the viewpoint of caries preveation does not show any effect on the puberty age.

Key words: fluoride-contain in water, caries, menarche, body development

Introduction

The caries development inhibitory effect of fluorine can by now be regarded as unanimously proved (ADLER, 1970; BACKER DIRKS, 1971; TÓTH, 1979; MARTHALER, 1979; JOHANSEN and OLSEN, 1979). There are also data on the fact that the daily optimal fluorine intake — which for example can be ensured with drinking water containing 1 mg/l fluoride — does not have any harmful effects on the human organism. These data, however, originate from the studies of such populations which live in geographical and economic environments different from that of Hungary (MCCLURE, 1944; MCCAULEY and MCCLURE, 1954; SCHLESINGER et al. 1956; Ministry of Health, 1962; SMITH, 1962; TRUSWELL, 1966; HODGE, 1968; BACKER DIRKS et al. 1969; BACKER DIRKS, 1971; ERICSSON, 1974; BINDER, 1974; MURRAY, 1976).

The number of human studies from Hungary related to the general effect of fluorine is relatively low (BARTHA, 1956; STRAUB and SZÜLE, 1956; ADLER, 1957; TÓTH et al. 1975).

If it is wished to apply fluoride in masses in the interest of preventing caries, this could only be done so with safety. To support the safety of prevention with fluoride it must be proved that the consequences drawn from studies carried out in different parts of the world also stand for the Hungarian conditions.

When evaluating the general effect of fluorine the daily total amount of fluoride reaching the circulation must be taken into account (COOK, 1973; NEWBRUN, 1975; MYERS, 1978). Although this amount decisively depends on the fluoride content of drinking water (TÓTH, 1975), every possible source, the living conditions, alimentary habits, individual variations of absorption, should also be considered which may dif-

fer in each case separately even besides the same fluoride concentration of drinking water.

In case the long-standing consumption of the daily fluoride content optimal in point of view of caries prevention would harmfully influence the normal physiological processes of the human organism, this should also be reflected in the state of health and development of the given population even between Hungarian circumstances.

To become familiar with this question more closely, a study was carried out on the somatic maturity of school-aged children from two Hungarian settlements where the fluoride concentration of the drinking water showed significant variations.

Our experiences on the bone development and body maturation of these two child populations have been published elsewhere (FAZEKAS et al., under publication, FAZEKAS et al., 1984). In this paper we should like to report on our results regarding the menarcheal age and reflecting the physiological maturation of the children.

Materials and Methods

In selecting the samples we were conducted by the basic viewpoint that the Hungarian popula-on to be studied should be such which regularly and long-standingly consumes drinking water containing fluoride of about 1 mg/1, held to be optimal between temperate climatic conditions. It was also our aim that the samples be suitable for statistic analysis.

In Hungary the fluoride concentration of drinking water is low in general. We only know of one settlement having higher population where the fluoride concentration of the drinking water is proved to be of optimal amount since years. This settlement is Kunszentmárton, a large village constituting an administrative division. Here the drinking water contains 1.09 mg of fluoride per litre.

The population of the settlement is 12 599 according to the data of the census taken in 1980. The caries preventive effect of the regular fluorine consumption is well reflected in the population's state of teeth (ADLER et al., 1950; ADLER and POLCZER, 1963; TÓTH, 1970; TÓTH et al., 1978).

Kiskunmajsa (population in 1980: 13 419) was chosen as control, a settlement where the drinkink water has low fluoride concentration (0,17 mg/1), but which is similar in other viewpoints to Kunszentmárton.

669 boys and 711 girls were studied in Kiskunmajsa and 617 boys and 589 girls in Kunszentmárton. The twins and gypsy children belonging to another ethnic group were left out of the evaluation.

During the course of the studies carried out at the schools, the personal data were recorded and dressed to underwear, the measurements of the body weight, body height, normal breast circumference and in case of the girls, the crystae width were taken according to the technique of Martin (MARTIN and SALLER, 1956). The information regarding first menstruation was also recorded in case of the girls on the data-collecting sheet used for the studies on the menarcheal age of girls in Csongrád county (FARKAS et al. 1983). During the processing of the data we calculated the parameters of the characteristics of the children divided into half-year age groups according to the decimal chronological table.

Results

Here we should only like to refer the fact that we did not find any essential changes in the studied characteristics of the morphological age and on it's base the body development of the two samples (FAZEKAS et al. under publication). The x-rays taken of the hands and the evaluations with TW2 method (TANNER et al. 1975) also did not manifest any differences in the time of bone development of the two studied populations.

We made inquiries concerning the time of the first menstruation in the case of 337 girls in Kunszentmárton, and 467 girls in Kiskunmajsa. Information is given in Tables 1 and 2 regarding the frequency the first menstruation, according to age groups.

The numerical method was used to determine the median (FARKAS, 1975) which gives the same result as the values obtainable by probit analysis.

Table 1. Distribution according to age gropus of the time of first menstruation of the girls of Kunszentmárton

Age group x	Total no. of cases n	Those menstruating from this		Probit of p. c. of those mentstruating P
		r	%	
10.0	15	1	6.67	3.50
10.5	30	—	—	—
11.0	26	2	7.69	3.58
11.5	41	6	14.63	3.95
12.0	27	8	29.63	4.46
12.5	48	16	33.33	4.57
13.0	34	23	67.65	5.46
13.5	35	26	74.29	5.65
14.0	17	13	76.47	5.72
14.5	14	14	100.00	—
15.0	6	6	100.00	—
15.5	9	9	100.00	—
16.0	12	12	100.00	—
16.5	11	11	100.00	—
17.0	4	4	100.00	—
17.5	4	4	100.00	—
18.0	2	2	100.00	—
18.5	1	1	100.00	—
19.0	—	—	—	—
19.5	1	1	100.00	—
	337	159	47.18	

Table 2. Distribution according to age groups of the time of first menstruation of the girls of Kiskunmajsa

Age group x	Total no. of cases n	Those menstruating from this		Probit of p. c. of those menstruating P
		r	%	
10.0	1	—	—	—
10.5	29	—	—	—
11.0	36	—	—	—
11.5	41	5	12.20	3.84
12.0	33	10	30.30	4.48
12.5	40	17	42.50	4.81
13.0	51	22	43.14	4.83
13.5	41	28	68.29	5.48
14.0	50	44	88.00	6.18
14.5	22	21	95.45	6.70
15.0	31	31	100.00	—
15.5	22	22	100.00	—
16.0	19	19	100.00	—
16.5	17	17	100.00	—
17.0	9	9	100.00	—
17.5	14	14	100.00	—
18.0	9	9	100.00	—
18.5	2	2	100.00	—
	467	270	57.82	

Tables 3 and 4 show the coincidence of the birth and menarche months in the two populations of girls.

In Kunszentmárton the median value of the menarche was found to be 12.779 years, and 12.79 years in the case of the Kiskunmajsa sample. The difference between the two medians is so slight (0.011 year) that it is not considered as substantial, since it should be taken into account that in case of the girls from the Hungarian and lowland settlements the difference found between the menarche median of the girls born as the first and third child, respectively was 0.37 year.

Accordingly, it could be said that even in the case of a more than six-fold difference in the fluoride content of drinking water, there are no essential changes observable in the time of puberty age of the girls.

In the followings such results of analysis regarding the time of first menstruation of the studied girl populations will be reported, which are not in direct connection with the basic question drafted in the objective, but may provide useful data to the special literature on the menarcheal age, according to our judgement.

The distribution of the monthly appearance of menarche is also usually studied, apart from the median. If it is presumed that the first menstruation of girls appears in an even distribution every month of the calendar year, then in each calendar month the first menstruation should appear in 8.33% of the girls.

Experimental data do not prove this assumption. Thus for example, in the case of the girls of Southern Hungary, in the Winter months and in August it's relative frequency is prominently high (FARKAS, 1962). In the case of these girls the first menstruation usually appears in the Winter months.

In the Kunszentmárton sample the highest frequency was found to be in January, while in Kiskunmajsa this was in August. According to this, the two girl communities do not differ from the cases experienced earlier. However, there was a difference observed, namely, that in both groups of girls the majority had their first menstruation in Summer (and not Winter).

Nevertheless, according to our opinion these observations do not mean substantial variations between the two samples, therefore it cannot be stated that the seasonal distribution of the menarche would differ from the ordinary on the probable effect of higher fluorine content in drinking water.

Examples can also be frequently found for studies carried out on the coincidence of birth and menarche moths. VALŠÍK, pointed out that there is no mathematical correlation betwen the two events (VALŠÍK, 1953). Despite this, author also found that generally the frequency that the adolescent menstruates for the first time in the same month as her birth is between 8—15%. VALŠÍK also referred to the fact that the size of the studied settlement may influence the frequency of coincidence, too (VALŠÍK, 1953). This is probably in accordance with the fact that in large cities the births are distributed more uniformly in the various months of the year than in villages where the habits of marriage are regulated better. Due to this, the seasonal distribution of births also varies.

This assumption is also supported by our previous data, since in the case of the girls of Szeged city having 200.000 inhabitants, the coincidence of the birth and menarche months was found to be 13.49% and 8.65% in the villages being in the neighbourhood of Szeged (FARKAS, 1962).

In the case of the girls of Kunszentmárton the monthly coincidence of the two events was observed to be 7.55%, and 12.64% in the case of the girls of Kiskunmajsa. The difference, however, can by no means be explained by the variations in size of the settlements, but rather by sociological causes not studied by us.

Table 3. Coincidence of the month of menarche and month of birth in the case of girls of Kunszentmárton

Month of birth			Month of menarche												Together		
			Spring			Summer			Autumn			Winter					
			III.	IV.	V.	VI.	VII.	VIII.	IX.	X.	XI.	XII.	I.	II.			
	Spring	III.	—	5	1	—	2	5	3	—	1	2	3	—	22	13.8%	46
		IV.	1	1	1	—	1	3	1	—	—	1	1	3	13	8.2%	29%
		V.	—	1	1	1	—	2	1	—	1	2	2	—	11	6.9%	
	Sommer	VI.	—	2	1	1	—	2	—	1	—	1	4	—	12	7.5%	
		VII.	1	1	1	—	1	2	2	—	1	—	2	1	12	7.5%	36
		VIII.	2	—	1	1	1	3	1	1	1	—	—	—	11	6.9%	22%
	Autumn	IX.	1	1	1	1	3	1	—	1	—	1	6	—	16	10.1%	35
		X.	—	—	—	—	3	—	1	—	2	—	2	1	9	5.7%	22%
		XI.	—	1	—	1	2	1	2	1	—	—	2	—	10	6.3%	
	Winter	XII.	1	2	—	—	—	—	2	1	1	2	1	1	11	6.9%	43
		I.	—	—	—	3	4	2	1	2	1	1	1	1	16	10.1%	27%
		II.	—	—	—	3	1	1	2	1	—	3	3	2	16	10.1%	
			6	14	7	11	18	22	16	8	8	13	27	9			
Total			3.8%	8.8%	4.4%	6.9%	11.3%	13.8%	10.1%	5.0%	5.0%	8.2%	17.0%	5.7%	159		
			27 17%			51 32%			32 20%			49 31%					

Table 4. Coincidence of the month of menarche and month of birth in the case of girls of Kiskunmajsa

birth of Month			Month of menarche												Together		
			Spring			Summer			Autumn			Winter					
			III.	IV.	V.	VI.	VII.	VIII.	IX.	X.	XI.	XII.	I.	II.			
	Spring	III.	2	3	1	5	—	3	1	2	4	—	4	2	27	10.0%	71
		IV.	1	2	1	—	2	4	—	—	2	2	4	—	18	6.7%	26%
		V.	1	2	1	3	6	3	2	1	3	2	2	—	26	9.7%	
	Summer	VI.	2	—	3	1	2	2	2	2	—	—	1	—	15	5.6%	55
		VII.	—	—	1	1	3	3	1	2	1	4	4	3	23	8.6%	21%
		VIII.	—	2	—	1	3	3	—	1	2	3	2	—	17	6.3%	
	Autumn	IX.	1	—	1	6	1	5	6	2	3	2	1	2	30	11.1%	73
		X.	1	2	—	5	3	2	5	1	—	—	1	—	20	7.4%	27%
		XI.	1	3	1	3	3	4	1	4	1	1	1	—	23	8.6%	
	Winter	XII.	2	1	—	8	1	6	1	—	1	6	—	1	27	10.0%	70
		I.	1	—	3	2	2	2	1	—	2	1	4	2	20	7.4%	26%
		II.	2	—	2	2	3	2	—	2	3	—	3	4	23	8.6%	
Total			14	15	14	37	29	39	20	17	22	21	27	14			
			5.2%	5.6%	5.2%	13.8%	10.8%	14.5%	7.4%	6.3%	8.2%	7.8%	10.0%	5.2%			269
			43 16%			105 39%			59 22%			62 23%					

Table 5. Empiric relative frequencies in the case of Hungarian girls

4—6	4—5	4—4	4—3	4—2	4—1	4	4+1	4+2	4+3	4+4	4+5	4+6	Sample size
Kunszentmárton													
101.8	88.3	132.4	94.6	66.2	110.3	110.3	88.3	147.2	165.6	66.2	120.4	101.8	159
Kiskunmajsa													
93.4	140.0	80.0	97.4	172.5	72.3	65.9	118.0	131.8	97.4	112.1	106.7	93.4	269
Western Hungary													
90.6	89.0	95.1	99.9	94.8	104.5	137.5	106.1	92.2	98.0	94.6	97.8	90.6	8255
Southern Hungary													
102.0	84.6	90.9	97.2	100.9	99.0	134.9	105.3	86.8	97.9	99.8	100.5	102.0	3247

A more realistic view could be gained if the empiric occurrence is compared with the monthly, expectable average occurrence obtained after dividing by 12 the total element numbers of the samples. In such a way the changes observable in the case of the different samples could be determined, compared to the average frequency.

The average frequency (rounded of) in the case of the Kunszentmárton sample was 159/12 = 13, and 269/12 = 22 in case of Kiskunmajsa.

The appropriate empiric relative frequency can be obtained if the absolute frequency is expressed in the percentage of the average frequency.

Table 5 shows comparisons between the samples of Kiskunmajsa, Kunszentmárton, Western Hungary and Southern Hungary. The column marked „n" indicates the empiric relative frequency of the coincidence of the birth and calendar months. In the columns n + 1, n+2..., n+6 these relative frequencies are related to such cases in which the first menstruation appeared 1, 2,..., 6 months later compared to the month of birth.

It can be seen from the Table that the coincidence of the months of birth and menarche and the percental occurrence of the further variations, resp. show changes according to samples. The higher variability observable in our samples may also evidently be influenced by the relatively low element numbers therefore it would not be correct to draw far-reaching conclusions from it.

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Address of the authors:
DR. GY. FARKAS
Department of Anthropology
A. J. University, H-6701 Szeged
P.O. Box 660, Hungary
DR. A. FAZEKAS
Clinic of Dental and Oral Surgery,
Medical University of Szeged
6720 Lenin krt. 64. Hungary
DR. E. SZEKERES
Public Health and Epidemiology
Station of County Csongrád, Szeged
6726 Derkovits fasor 5—7, Hungary

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STUDIES ON THE MENARCHEAL AGE OF THE GIRLS OF COUNTY CSONGRÁD (SOUTHERN HUNGARY)

GY. FARKAS, P. HUNYA, I. HERENDI and ERZSÉBET SZEKERES

Department of Anthropology and Kalmár Laboratory of Cybernetics, Attila József University; Public Health and Epidemiology Station of County Csongrád, Szeged
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Abstract

A review is given of the method of a research work carried out in county Csongrád (Southern Hungary) between the period 1981 and 1984. Using the literature of this topic, authors report on the purpose of the research: the joint studying of the physical, social and biological factors influencing the puberty of girls. The study comprises 20.000 girls from the age of 10—18 years, and also determines the mainer body measurements of the boys of similar age.

As the first partial result of this research, authors report on the parameters of four body measurements of nearly 7000 girls of Szeged (body height, body weight, normal breast circumference, bicristal width) as well as the menarcheal age.

Key words: menarche, body development, girls, Hungary.

Introduction

It could be said on the basis of reviewing the international literary data that studies have been carried out on the time of the first menstruation of girls and the factors influencing it is many countries, from several viewpoints. The literature of the topic is extremely large. Most of the experiments, however, only focussed on the determination of the median in the studies aiming to determine the menarcheal age. It also became evident on the basis of the observations so far that this age could be related to several factors.

These influencing factors could be divided into four groups:
1. social factors, 2. biological factors connected with the body endowments, 3. naturally, and 4. other factors (FARKAS, 1980.) However, the influence of these factors on the time of maturation could not be made clear as yet.

The opinions regarding the effect of the factors could also be divided into three groups:

1. The effects of certain factors are unanimously presumed, but the degree and trend (negative or positive) of the effects are not known completely yet.

Thus, it is known that radioactive radiation has retarding effect (BURROW et al., 1965), nevertheless, its degree — since luckily we do not have enough data — is not known yet.

Similarly, it is also known that the first menstruation during the whole calendar year is not uniform in a population, but seasonal variations can be observed (BOJLÉN and BENTZON, 1968, 1971; BREIPOHL, 1938; BURREL et al. 1961; FARKAS, 1971), however, the relative frequency regarding each calendar month may differ even within a population, although the coincidence of the months of birth and menarche is almost identical in the same population (EIBEN and BODZSÁR, 1970; FARKAS, 1971). ENGLE and SHELESNYAK (1934) were the first to call the attention to the seasonal variations — which are related with all probability to the habits and time of marriage and consequently, the time of birth, too.

The genetical factors also determine the period of puberty (CHERN, 1973; TISSERAND-PERRIER, 1953).

During the past 100 years the time of puberty pushed to a more and more earlier age (acceleration), which has been observed by many authors (AMUNDSEN and DIERS, 1969, 1973; BACKMAN, 1948), and which can even be observed during a few decades (FARKAS, 1969), but the degree of acceleration may vary.

2. Such effects could be ranked into the second group, which produced different opinions.

Certain authors who studied the occupation of the parents found that the daughters of intellectual parents reach the age of puberty earlier than those of manual worker parents (BARISIĆ and GAVRILOVIĆ, 1974; RICHTER, 1973; BODZSÁR, 1975). Others did not find such relationships (BER and BROCINER, 1964; ROBERTS and DANN, 1967; ROBERTS et al. 1971). We ourselves have found this relationship unanimously on the basis of the occupation of fathers, however, this did not prove to be true in the case of mothers.

It is a generally accepted opinion that the girls living in the Northern regions become mature later than those living in countries of warmer climate. Furthermore, different median can be observed even with the slight modification of the height above sea level (FARKAS, 1979a), while in other areas no connections could be demonstrated between the geographical position and the menarche median (GRIMM, 1958; ŁASKA-MIERZEJEWSKA, 1970).

The financial situation (average income) of the family plays a role as a demonstrable effect in certain countries (AW and TYE, 1970), while others contradict the significance of this, or at least the connection between the time of puberty and the economical situation of the family is not unambiguous (BAI and VIJAYALAKSHMI, 1973).

Similar uncertainty can be experienced in the judgement of the effect of other factors, also, like the quality composition of food and the mode of meals (DREIZEN et al., 1967; CARFAGNA et al., 1973; ŠKERLJ, 1947), the body development (RICHTER, 1973; WINICK, 1975; FARKAS and SZEKERES, in press), the number of brothers and sisters (FARKAS et al., in press; ŠTUKOVSKÝ et al. 1967; SOENDEROP et al. 1961; SCOTT, 1961; ROBERTS, 1977), and the climate (FOLL, 1958; FARKAS, 1979a).

3. Finally there are such opinions, too, according to which the time of the first menstruation shows complete variation (ROBERTS and DANN, 1967), making its dependence on the afore-mentioned factors practically unrecognizable.

In all probability the opinion of SCHWENK stands closest to the truth, according to which author the causes producing menarche are not known well enough as yet, but it is very likely that the variations in the amount of oestrogens also play a large role in this (SCHWENK, 1965).

With the above cited opinions we only wished to bring a few examples as evidence for those reported above, emphasizing that this is only a fragment of the huge literary data on the topic, the complete listing of which is unnecessary and impossible in this report.

These opinions could not even be definitely cleared by the observations in Hungary so far, even though a few studies had been reported on even earlier (BOTTYÁN et al., 1963; BODZSÁR, 1975; EIBEN, 1972).

According to our opinion the followings are the main causes for this:

a) the collection of data is generally done by status quo method (one single surveying and questioning) and there are only few observations originating from so-called longitudinal samples. (It is a fact, that this latter means a close to 10 years' research work in a child community).

b) authors mostly analyse their study material collected with the status quo method according to only one or a few viewpoints, although the studying of several factors can also be easily carried out in case of a single sampling, with the spending of relatively short time.

The collections of data accomplished by one of the authors since 1958 in Southern Hungary are also connected with these international researches, of which several reports have already been published (FARKAS, 1962; BOTTYÁN et al. 1963; FARKAS, 1963, 1964, 1969, 1970, 1971, 1975, 1976, 1979a, 1979b, 1980; FARKAS and SZEKERES, in press).

The contradictions of the literary data as well as the practical experiences induced us to study the effects of possibly several factors in the case of one and the same child community, in the frame of larger sampling. On this basis we started a research work in 1981, the methods and first partial results of which are reported in the followings.

Materials and methods

Our aim is to initiate in our study as many as possible girls of county Csongrád ranging from the age of 10—18 years. This is wished to be achieved in such a way that the collection of data is carried out in every primary and secondary school where the pupils are mostly girls. Only those are left out of the study, who are sick during the course of the surveying, are absent from school, or do not wish to take part in the supplying of data, which is voluntary. According to our judgement about 20.000 girls from the county will take part in the study, which means nearly 95% of the girls from the given age.

The 10—18 years of age limits were determined from a methodological point of view, since the evaluations are carried out by probit analysis, for which it is necessary to know the age when the girls do not menstruate yet, and also the age when 100% of them do. According to our experiences this falls between the above mentioned ages in case of the Hungarian girls. For the data collection such a study sheet was constructed (see enclosed) which contains the questions grouped according to topics and numbered.

A brief information for the parents and teachers is given of the purpose of the study on the data collecting sheet.

The following questions were ranged into groups (here we are also giving the serial number of each question in parentheses):

Data of identification. — serial number (1), permanent address (2), place of data collection (3), time-point of data collection (4), date of birth of pupil (5), occupation of father (6), occupation of mother (7), number of living and deceased brothers and sisters of pupil (8), how many brothers and sisters were born before the pupil (9), the results of her latest school certificate (10), the number of several organic diseases so far (11), number of operations (12).

Somatical data. — body weight (13), body height (14), normal breast circumference (15), bicristal width (16), development of pubic hair (17), development of underarm hair (18), development of breast (19).

Questions concerning menarche. — does the pupil have menstruation (20), if yes, exactly when (21), is it regular (22).

Data of parents. — year of birth of father (23), place of birth of father (24), highest school qualification of father (25), time of birth of mother (26), place of birth of mother (27), highest school qualification of mother (28).

Other questions. — weight at birth of pupil (29), does the pupil have a twin, if yes what is the twin's name (30), the time of first menstruation of mother (31), have the parents talked with their child about sexual questions (32), the member of the family filling out the form (33), what type of school does the pupil attend (34), colour of eyes (35), colour of hair (36).

The questions number 1, 3, 4, 13—19, 35—36 are filled out during the course of examination. The rest of the questions are requested to be filled out by the parents in such a way that the pupil takes the form home and asks for answers to the encircled questions from her parents.

The form is then taken back to school, partially filled out and the body measurements taken according to the specifications of MARTIN are written on the form (MARTIN and SALLER, 1956). The measurements are determined using anthropometer, scales measuring with 50 g exactness, steel measuring tape, and calipers (questions No. 13—16.). Each measurement is generally taken by the same person. The specifications of TANNER (1962) are followed in determining the secondary sexual characteristics (questions No. 17—19), the colours of the eye and hair are determined by the colour scales of FISCHER-SALLER and MARTIN, respectively.

JÓZSEF ATTILA TUDOMÁNYEGYETEM
EMBERTANI TANSZÉKE, SZEGED

CSONGRÁD MEGYEI KÖJÁL GYERMEK- ÉS
IFJÚSÁGEGÉSZSÉGÜGYI OSZTÁLYA, SZEGED

Iskola megnevezése (bélyegzője)

Tizenévesek menarche adatgyűjtő lapja

Tisztelt Szülők!

A gyermek egészséges testi fejlődésének érdekében szeretnénk az eddiginél pontosabban megismerni a serdülőkor változásait. Ennek érdekében nagyszámú adatgyűjtést kívánunk végezni. Kérjük Önöket, hogy a kérdésekre adott pontos válaszukkal segítsék elő munkánkat, amivel a jövő nemzedék harmónikus fejlődéséhez közvetve, ily módon hozzájárulhatnak. Fáradozásukat előre is köszönjük.

A bekarikázott számú kérdéseknél csak a kipontozott (...) részhez írják be válaszukat, a négyzetek és a bekeretezett szövegrészek maradjanak üresen. A 20., 22., 32., 34. számú kérdéseknél a megfelelő szót szíveskedjenek aláhúzni. Az adatgyűjtőlap kitöltése önkéntes. Az adatok felhasználása kizárólag tudományos célra történik.

AZONOSÍTÁSI ADATOK

1. Sorszám: ..... 1—6
2. Állandó lakhely (város, község):
..... megye
(0) 100—200 ezer (1) 50—100 ezer (2) 10—50 ezer (3) 5—10 ezer (4) 5 ezer alatt (5) 200 ezer felett 7
3. Adatgyűjtés helye (város, község):
..... megye
(0) 100—200 ezer (1) 50—100 ezer (2) 10—50 ezer (3) 5—10 ezer (4) 5 ezer alatt (5) 200 ezer felett 8
4. Adatgyűjtés ideje: 198.... év .... hó .... nap 9—14
5. A tanuló szül. ideje: 19.... év .... hó .... nap 15—20
6. Apa foglalkozásának pontos megnevezése:
(0) ifm (1) mfm (2) efm (3) szdfv (4) szdkv (5) ny (6) mh (7) e 21
7. Anya foglalkozásának pontos megnevezése:
(0) ifm (1) mfm (2) efm (3) szdfv (4) szdkv (5) ny (6) mh (7) e (8) htb 22
8. A tanuló testvéreinek száma (a tanulón kívül):
élő fiú ..... meghalt fiú .....
élő leány ..... meghalt leány ..... 23—26
9. A tanuló hányadiknak született: .... 27—28
10. A tanuló legutóbb évvégi bizonyítványának átlageredménye: ..... 29—30
11. A tanuló átélt súlyosabb szervi megbetegedései: ..... 31—32
12. A tanulónak milyen műtétei voltak: 33

SZOMATIKUS ADATOK

13. Pondus corporis (ts) .....kp 34—36
14. Altitudo corporis (tm) .....cm 37—39
15. Circumferentia thoracis (hmk).....cm 40—42
16. Latitudo pelvis (csz) .....cm 43—45
17. Pubes: 46
18. Regio axillaris: 47
19. Mamma: 48

MENARCHE ADATAI

20. Volt-e már a tanulónak vérzése (első menstruációja): IGEN—NEM (megfelelő szót aláhúzni) 49
21. Ha volt vérzése, mikor: 19.... év .... hó .... nap 50—55
22. Rendszeres-e a vérzése (menstruációja): IGEN—NEM (megfelelő szót aláhúzni) 56

SZÜLŐK ADATAI

23. Apa születési éve: 19.... év .... hó .... nap 57—58
24. Apa születési helye (0, 1, 2, 3, 4, 5): 59
25. Apa legmagasabb iskolai végzettsége: .....
(0) nem fejezte be a 8 általános iskolát (1) 8 általános iskola (2) szakmunkásképző (3) középiskola (4) főiskola vagy egyetem 60
26. Anya születési ideje: 19.... év .... hó .... nap 61—62
27. Anya születési helye (0, 1, 2, 3, 4, 5) 63
28. Anya legmagasabb iskolai végzettsége: .....
(0) nem fejezte be a 8 általános iskolát (1) 8 általános iskola (2) szakmunkásképző (3) középiskola (4) főiskola vagy egyetem 64

EGYÉB ADATOK

29. A tanuló születési súlya: .....gr 65—68
30. A tanulónak van-e ikerpárja, ha igen, írja fel a nevét: 69
31. Az anya első vérzésének (első menstruációjának ideje): 19.... év .... hó .... nap 73—75
32. Beszélgettek-e szexuális kérdésekről a szülők a leányukkal: IGEN—NEM (megfelelő szót aláhúzni)
33. Ki töltötte ki a kérdőívet: .....
(0) anya (1) apa (2) anya a leányával (3) apa a leányával (4) nagyszülő (5) nagyszülő a tanulóval (6) nincs válasz
34. A tanuló milyen iskolában tanul:
(0) általános iskola (1) szakmunkásképző (2) középiskola 76
35. Color oculi (szsz): ..... 77—78
36. Color capilli (hsz): ..... 79—80

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During the course of the measurings, the exactness of the filling out of the forms is checked.

The evaluation of the data is carried out with R-40 type computer using the Osiris programe. The data collecting forms are coded for this procedure.

The settlements are divided into six categories on the basis of the number of the population of the living place and place of data collecting: settlements having more than 200,000 inhabitants; 200—100 thousand; 100—50 thousand; 50—10 thousand; 10—5 thousand inhabitants; less than 5 thousand inhabitants.

Taking a unified classification system as a basis, the occupations of the parents are divided into 9 categories: industrial manual workers; agricultural manual workers; manual workers in other fields; intellectuals with higher qualification (university, college); intellectuals with secondary qualification; pensioners; others; home workers; and those pupils whos either parents have died are ranked into a separate group.

On the basis of the parents' school qualification the following groups are differentiated: those who did not finish their primary school studies; those who had finished their primary school studies; those who received vocational training; those who have secondary school; college or university qualification.

Finally, the type of school is determined which the children attend: primary school; vocational school training (3 years); secondary school (4 years).

Since the punch card used for the R-40 type computer is prepared for 80 code signs, the question No. 33 is not coded (it would be the 81, code).

After taking the coded data on the punch card and following the processing in the computer the estimates received by the computer are checked until the errors are completely eliminated. Durring this course even those data can be deleted which are not true (e. g. wrong data are given).

The 34 different types of information give the possibility to have them evaluated independently as well as combined with each other. With this procedure further information can be gained. As an example, the pupils could be grouped according to the number of brothers and sisters, the parents' school qualification, the age differences between mother and father, etc., and in compliance with this we are able to determine the changes in the menarcheal median.

As it can be seen, the questions attempt to comprehend the viewpoints appearing in the international literature.

The studied pupils are divided into 8 groups before the evaluation by the computer as follows: girls and boys, resp. of Szeged; girls and boys, resp. of county Csongrád (the previous two groups are also comprised in this group); girls and boys, resp. from other counties; gypsy girls and boys, respectively.

According to our original intention the research would only have limited to the pupils of county Csongrád, however, during the course of the study it turned out that children from other counties also attend the schools in county Csongrád (schools giving vocational training and secondary schools), These had to be separated from those of county Csongrád, and will belong to a national comparative study, which will be amplified by data collection from other counties.

The students are divided into half-year age groups in every case and this is also performed with the help of a computer using the decimal life-table (see enclosed).

Apart from the girls, the boys are also measured in the case of coeducational schools, as this does not mean particular difficulty. In the case of boys, the time of birth, time of data collecting, address, body height and weight, as well as the normal breast circumference are recorded.

For our work we have received moral and financial support from the Ministry of Health and Ministry of Education.

Besides the authors, MRS. MARIANNE KALMÁR, assistant; MRS. ANNA SZEDERKÉNYI, statist; and GYÖRGY NÉMETH, also performing the coding, take part in the research work.

Results

The work was started on the 23rd February, 1981 and the measurings are taken always by the same team. A total of 12,729 girls and 7537 boys were measured till July 31st, 1982. From these, the evaluation of the body measurements of 7000 girls and 2700 boys of Szeged has been accomplished by now. Tables 1.—4. show the important parameters of the body weight, height, normal breast circumference and bicristal width of the girls.

The findings until now, which are firstly related to the comparisons of the earlier data with the physical maturity of the 10 to 14.5 years old girls and boys of Szeged are summarized in a separate report to be published in this volume of Acta Biologica Szege-

diensis (FARKAS, 1983). Here, we should only like to mention in brief that the body height and weight of the children belonging to the age group referred to above still show a lag compared to the averages of 1966/67.

The menarcheal median is 12.77 years on the basis of the present data, being practically unchanged compared to the median of 1966/67.

Finally, we should like to mention that related to this research, we have also studied the relationship between the fluoride content of drinking water and physical maturity, which study will partly be published in other periodicals (FAZEKAS et al., in press, FAZEKAS et al., 1984), and partly in this volume (FARKAS et al., 1983).

Decimal age table of I. B. P.

NAP	JAN 1	FEB 2	MAR 3	APR 4	MAY 5	JUN 6	JUL 7	AUG 8	SEP 9	OCT 10	NOV 11	DEC 12
1	000	085	162	247	329	414	496	581	666	748	833	915
2	003	088	164	249	332	416	499	584	668	751	836	918
3	005	090	167	252	334	419	501	586	671	753	838	921
4	008	093	170	255	337	422	504	589	674	756	841	923
5	011	096	173	258	340	425	507	592	677	759	844	926
6	014	099	175	260	342	427	510	595	679	762	847	929
7	016	101	178	263	345	430	512	597	682	764	849	932
8	019	104	181	266	348	433	515	600	685	767	852	934
9	022	107	184	268	351	436	518	603	688	770	855	937
10	025	110	186	271	353	438	521	605	690	773	858	940
	1	2	3	4	5	6	7	8	9	10	11	12
11	027	112	189	274	356	441	523	608	693	775	860	942
12	030	115	192	277	359	444	526	611	696	778	863	945
13	033	118	195	279	362	447	529	614	699	781	866	948
14	036	121	197	282	364	449	532	616	701	784	868	951
15	038	123	200	285	367	452	534	619	704	786	871	953
16	041	126	203	288	370	455	537	622	707	789	874	956
17	044	129	205	290	373	458	540	625	710	792	877	959
18	047	132	208	293	375	460	542	627	712	795	879	962
19	049	134	211	296	378	463	545	630	715	797	882	964
20	052	137	214	299	381	466	548	633	718	800	885	967
	1	2	3	4	5	6	7	8	9	10	11	12
21	055	140	216	301	384	468	551	636	721	803	888	970
22	058	142	219	304	386	471	553	638	723	805	890	973
23	060	145	222	307	389	474	556	641	726	808	893	975
24	063	148	225	310	392	477	559	644	729	811	896	978
25	066	151	227	312	395	479	562	647	731	814	899	981
26	068	153	230	315	397	482	564	649	734	816	901	984
27	071	156	233	318	400	485	567	652	737	819	904	986
28	074	159	236	321	403	488	570	655	740	822	907	989
29	077		238	323	405	490	573	658	742	825	910	992
30	079		241	326	408	493	575	660	745	827	912	995
31	082		244		411		578	663		830		997
	1 *JAN*	2 *FEB*	3 *MAR*	4 *APR*	5 *MAY*	6 *JUN*	7 *JUL*	8 *AUG*	9 *SEP*	10 *OCT*	11 *NOV*	12 *DEC*

Table 1. Parameters of body height. Girls

Age	n	$\bar{x}$	s	w
10.0	38	142.63	7.25	127.9—158.8
10.5	395	141.50	6.61	124.2—160.0
11.0	487	144.80	7.35	126.7—166.8
11.5	480	147.77	7.48	128.9—172.0
12.0	473	151.03	7.20	127.8—172.5
12.5	474	153.73	6.94	134.8—178.7
13.0	528	156.01	6.88	134.6—180.1
13.5	466	158.33	6.85	126.7—177.1
14.0	392	159.32	6.04	124.0—174.2
14.5	377	159.82	5.92	132.2—174.4
15.0	512	160.07	6.04	141.3—180.1
15.5	485	161.03	6.36	140.7—178.3
16.0	475	160.39	6.07	128.5—181.3
16.5	407	160.71	7.04	125.4—190.0
17.0	365	161.17	6.43	138.5—178.1
17.5	244	160.94	5.94	147.7—176.1
18.0	184	162.18	5.39	149.8—176.4
18.5	87	161.42	7.15	149.0—180.4
19.0	10	162.63	5.91	154.7—171.9
	6879			

Table 2. Parameters of body weight. Girls

Age	n	$\bar{x}$	s	w
10.0	38	35.44	6.71	25.0—53.4
10.5	395	35.70	7.75	21.2—77.4
11.0	487	37.53	8.82	22.4—82.9
11.5	480	39.68	8.71	22.2—70.7
12.0	473	42.97	9.28	25.3—75.2
12.5	474	45.80	9.72	24.2—84.8
13.0	528	47.29	9.72	27.3—90.3
13.5	466	50.43	11.06	25.5—96.4
14.0	392	51.84	8.89	28.4—91.3
14.5	377	53.12	9.68	31.0—93.9
15.0	512	54.75	9.28	33.1—90.9
15.5	485	55.63	9.30	34.7—96.3
16.0	475	55.17	8.54	35.7—87.2
16.5	407	55.86	8.52	29.4—86.2
17.0	365	56.47	8.71	35.5—99.8
17.5	243	55.86	8.14	40.3—89.1
18.0	184	57.14	8.13	43.2—86.1
18.5	87	56.21	8.58	32.7—78.9
19.0	10	55.81	5.13	47.9—65.3
	6878			

Table 3. Parameters of normal chest circumference. Girls

Age	n	$\bar{x}$	s	w
10.0	38	66.84	5.58	59—84
10.5	395	67.33	7.24	53—97
11.0	487	68.72	7.78	55—103
11.5	480	70.63	7.75	53—99
12.0	473	73.59	8.05	57—103
12.5	474	76.38	8.28	54—105
13.0	528	77.94	8.31	60—109
13.5	466	80.40	9.20	61—105
14.0	392	81.66	7.34	63—113
14.5	377	83.30	7.99	59—111
15.0	512	84.66	7.41	66—114
15.5	485	85.49	7.40	69—115
16.0	474	85.50	6.92	72—110
16.5	407	85.57	6.77	53—110
17.0	365	86.61	6.99	70—113
17.5	244	86.28	7.27	65—112
18.0	184	86.49	7.17	59—111
18.5	87	86.40	6.67	70—105
19.0	10	85.20	3.22	81—91
	6878			

Table 4. Parameters of bicristal width. Girls

Age	n	$\bar{x}$	s	w
10.0	38	22.22	1.50	20.0—26.7
10.5	395	22.21	1.56	18.2—28.1
11.0	487	22.79	1.82	18.0—31.2
11.5	480	23.29	1.82	18.2—29.5
12.0	473	24.09	1.80	19.2—29.6
12.5	474	24.70	1.93	18.8—31.5
13.0	528	25.25	1.79	20.9—32.4
13.5	466	25.73	1.85	19.1—33.1
14.0	392	25.97	1.69	20.0—32.0
14.5	376	26.55	1.81	20.6—36.7
15.0	512	26.82	1.64	22.7—31.8
15.5	482	26.97	1.71	20.1—33.4
16.0	473	27.04	1.58	22.2—32.0
16.5	407	27.19	1.65	22.5—35.0
17.0	361	27.38	1.57	22.2—32.4
17.5	244	27.34	1.70	21.0—33.1
18.0	184	27.59	1.56	23.5—31.6
18.5	87	27.39	1.71	22.7—31.0
19.0	10	28.23	1.61	25.5—31.0
	6869			

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Address of the authors:
DR. GY. FARKAS
Department of Anthropology
A. J. University, H-6701 Szeged
P.O. Box. 660, Hungary
DR. P. HUNYA
DR. I. HERENDI
Kalmár Laboratory of Cybernetics
A. J. University, H-6701 Szeged
P.O. Box 428, Hungary
DR. ERZSÉBET SZEKERES
Public Health and Epidemiology
Station of County Csongrád, Szeged
6726 Derkovits fasor 5—7, Hungary

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CHANGES IN BODY MEASUREMENTS OF ADOLESCENT CHILDREN IN SZEGED, HUNGARY, BETWEEN 1958 AND 1981

GY. FARKAS

Department of Anthropology, Attila József University, Szeged
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Abstract

The averages in body weight, body height and normal breast circumference of 10—14.5 years old urban boys and girls were compared on the basis of four different measurements carried out at various time points. The observations are from single surveys. It is determined that between 1958/59 and 1966/67 the averages of the three body measurements increased in each age group, in the case of both sexes. According to the measurings in 1981/82 the increase in averages could only be observed in body height and weight, while the averages of the normal breast circumference were lower in 1981/82 than in the averages of 1966/67 in the majority of the age groups in both sexes.

This phenomenon was also observed in children of nursery age.

The menarche-median did not show any essential changes in the last 15 years.
Key words: body growth, menarche, acceleration.

Introduction

The supervision of the body growth of children is an everyday task. It is especially important in children living between approximately similar environmental conditions, where there is also a possibility to make conclusion of the general regularities on the basis of the data. Naturally, the major part of the examinations of growth following birth also comprises the examination of the adolescents, in which case the researchers also follow with attention the physiological maturation besides the body measurings.

Such examination of the children living in Szeged, a city in Southern Hungary having 200,000 inhabitants, was carried out in the years of 1958/59, 1961 and 1966/67 (FARKAS, 1961, 1966, 1969). A large part of these studies comprised mostly the examination of the factors influencing the puberty of girls (FARKAS, 1969, 1970, 1979a, 1979b, 1980a, 1980b). The results of these studies supported the observation of foreign researchers, according to which puberty is influenced by several environmental and endogenous biochemical factors (BARISIĆ and GAVRILOVIĆ, 1974; CHANG et al. 1967; ŁASKA-MIERZEJEWSKA, 1970; RICHTER, 1973; SIMELL, 1951; ŠTUKOVSKÝ et al. 1967; VALŠÍK and ŠTUKOVSKÝ, 1964), but also revealed newer relationships (FARKAS, 1979a, 1979b). According to our knowledge there has been no joint observation of these factors anywhere as yet in the case of one and the same child community, in the frame of larger material. Therefore we started a 3-years study in Csongrád county (Southern Hungary, county centre Szeged).

In the followings, in the frame of this study, the partial results of the data collection and measurements carried out in 1981/82 in Szeged are compared with the averages obtained earlier from the children of the same settlement.

Materials and methods

7000 girls and 4810 boys ranging from 10 to 19 years of age were measured in Szeged in the years 1981/82.

The measurements were taken with the general anthropometrical technique (MARTIN and SALLER, 1956), using anthropometer, scales measuring with 50g exactness, steel measuring tape and calipers.

The students were divided into half-year age groups on the basis of the decimal age group table, according to the formula of the year of age ± 3 months.

Using R-40 type computer and Osiris programme the most important parameters were determined according to characteristics. The arythmetic averages obtained in 1981/82 and 1966/67 were compared with two-sample t-test.

The parameters of the three body measurements originating from the two samples are shown on Tables 1.—6.

The growth curves designed on the basis of the arythmetic averages determined in the school years of 1958/59, 1966/67 and 1981/82 are observable on Figs. 1.—6.

It should be mentioned that the Tables contain only the parameters of the 10—14.5 years old children, therefore the total sample element number both in the case of boys and girls, does not reach the case number of the total boys and girls measured in 1981/82.

Results

On the basis of the data gained earlier it could be determined that the averages of body height, body weight and normal breast circumference of the children of Szeged showed an increase in both sexes and every age group compared to the previously obtained data (Figs. 1—6).

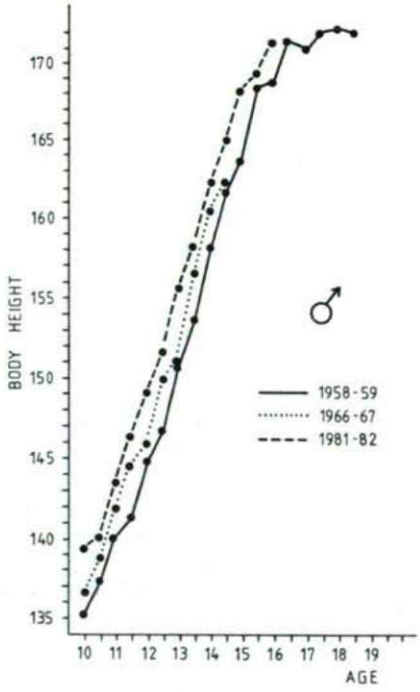

Figure 1. Growth curve of body height of the boys of Szeged.

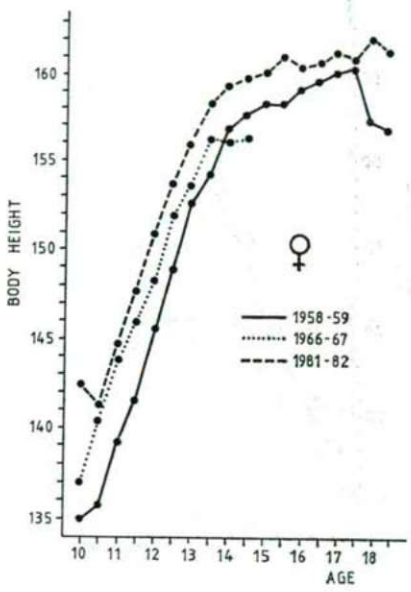

Figure 2. Growth curve of body height of the girls of Szeged.

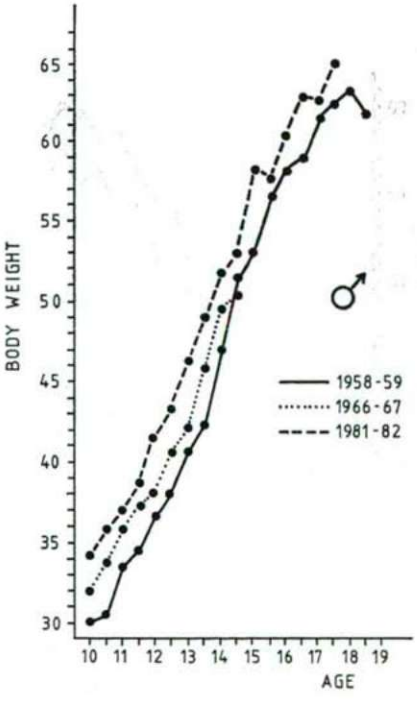

Figure 3. Growth curve of the body weight of the boys of Szeged.

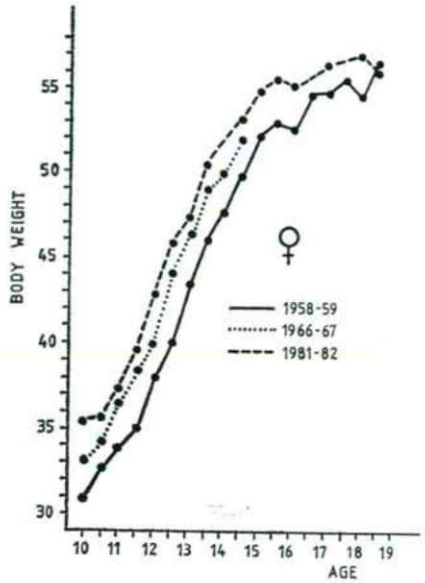

Figure 4. Growth curve of the body weight of the girls of Szeged.

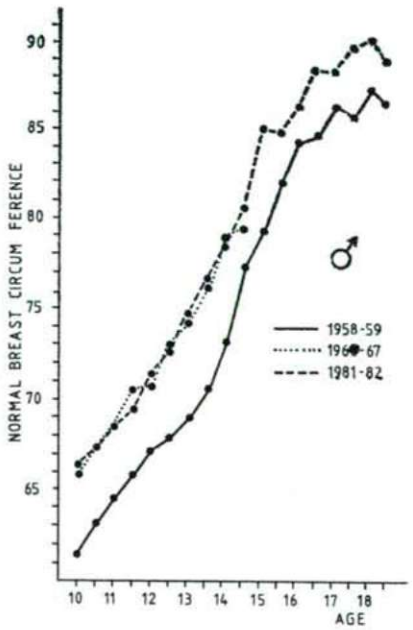

Figure 5. Growth curve of normal breast circumference of the boys of Szeged.

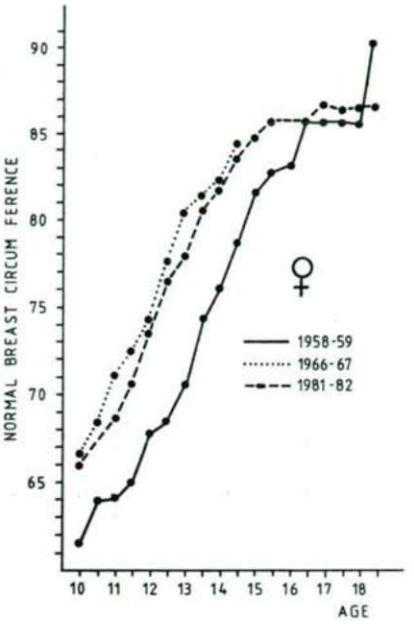

Figure 6. Growth curve of normal breast circumference of the boys of Szeged.

This acceleration phenomenon in Hungary was not only detectable in the case of the children in Szeged. The improvement of the living standards and hygienic conditions in Hungary after World War II can be mentioned as the unambiguous cause of this finding. Obviously, the high development in the medical attendance of children, among others, the decrease in the amount of child diseases, their milder course and prevention also play role in this.

The acceleration was observable in the puberty period of girls, too, since the menarche median of the girls of Szeged in 1958/59 was 13.20 years which decreased to 12.73 years by 1966/67 (FARKAS, 1969).

In connection with the acceleration the question naturally arises: how long can it last in the case of children living in a certain geographical region, under given socal-economic circumstances? The decision of this is only possible after the periodically carried out measurements. This was one of the causes for initiating another data collection in Szeged in 1981/82.

In Tables 1.—6. the averages of the 1981/82 measurements are compared with those of 1966/67. The averages of 1981/82 are also shown on Figs. 1.—6. In the last column of the mentioned Tables the differences between the averages of the two study periods are given, in such a way that in case the 1981/82 averages were lower than those of 1966/67, this was indicated by a negative sign. In the „t" column of the Tables the thickly set values indicate the differences of 95%, or the differences assertable with higher certainty.

On the basis of these Tables and Figures it is unanimously evident that the body height and weight averages of the boys and girls of Szeged between the age of 10—14.5 years are higher in every age group in 1981/82 than they were in 1966/67. Hovewer, the differences are not provable in every age group with statistic tests.

The higher stature averages obtained in 1981/82 in the 10, 11 and 14.5 years old boys and the 10.5, 11 years old girls cannot be proved by statistic methods. It was also experienced in the rest of the age groups that the latest measurement averages are higher than the earlier ones, but these are provable also by statistic procedure, on a level of 99% in the case of the girls — contrary to the boys. Therefore, there is a difference in this respect between the two sexes (Tables 1.—2.).

Table 1. The stature parameters of the boys of Szeged

1981/82			Age	1966/67			t	Diff.
n_1	$\bar{x}_1$	s_1		n_2	$\bar{x}_2$	s_2		
26	139.38	6.74	10.0	70	136.70	6.38	1.80	2.68
251	141.13	6.20	10.5	68	138.80	7.60	**2.61**	2.33
289	143.48	7.10	11.0	71	142.00	7.73	1.55	1.48
303	146.31	7.09	11.5	122	144.60	6.41	**2.31**	1.71
286	149.17	7.74	12.0	150	146.00	6.47	**4.29**	3.17
336	151.62	8.34	12.5	182	150.00	7.76	**2.16**	1.62
268	155.45	8.66	13.0	151	151.00	7.81	**5.23**	4.45
320	158.12	8.84	13.5	167	156.50	8.03	**1.98**	1.62
330	162.21	8.77	14.0	104	160.30	8.53	**1,95**	1.91
340	164.80	8.35	14.5	29	162.20	9.01	1.60	2.60
2749				1114				

Remark: In the „t" column the thickly set values indicate the differences provable by 95%, or by higher probability.

Table 2. The stature parameters of the girls of Szeged

1981/82			Age	1966/67			t	Diff.
n_1	$\bar{x}_1$	s_1		n_2	$\bar{x}_2$	s_2		
38	142.63	7.25	10.0	58	137.10	5.98	**4.07**	5.53
395	141.50	6.61	10.5	69	140.60	6.62	1.04	0.90
487	144.80	7.35	11.0	72	144.00	7.54	0.86	0.80
480	147.77	7.48	11.5	113	146.10	6.76	**2.17**	1.67
473	151.03	7.20	12.0	119	148.40	7.20	**3,56**	2.63
474	153.73	6.94	12.5	112	152.00	6.79	**2.38**	1.73
528	156.01	6.88	13.0	120	153.70	7.27	**3.29**	2.31
466	158.33	6.85	13.5	89	156.20	5.88	**2.75**	2.13
392	159.32	6.04	14.0	57	156.10	4.99	**3.84**	3.22
377	159.82	5.92	14.5	23	156.40	4.45	**2.72**	3.42
4110				832				

Remark: In the „t" column the thickly set values indicate the differences provable by 95%, or by higher probability.

Similar phenomenon was observable between the two sexes in the body weight averages, too. The averages of 1981/82 are higher both in girls and boys. At the same time, however, these differences can only be proved by statistic test in one age group in case of the girls (12 years of age), but in 5 age groups in case of the boys (10.5; 12—13.5 years of age). The provable level was 99% in case of both sexes (Tables 3.—4.). It seems, therefore, that the average values of the boys verifiably higher in several cases, were the results of more intensive increase in body weight, which also seems to be supported by the fact that between the 10—14.5 years the total differences in the case of the two samples were 24.85 kg in boys and 16.30 kg in girls.

Table 3. Body weight parameters of the boys of Szeged

1981/82			Age	1966/67			t	Diff.
n_1	$\bar{x}_1$	s_1		n_2	$\bar{x}_2$	s_2		
26	34.21	7.08	10.0	70	32.00	5.33	1.65	2.21
252	35.75	7.32	10.5	68	33.80	6.19	**2.01**	1.95
289	37.15	7.58	11.0	71	35.90	6.23	1.29	1.25
303	38.93	8.42	11.5	122	37.40	7.43	1.7.	1.53
286	41.47	8.93	12.0	150	38.10	7.29	**3.98**	3.37
336	43.33	9.61	12.5	182	40.70	7.91	**3.16**	2.63
268	46.31	9.80	13.0	151	42.20	8.27	**4.35**	4.11
320	49.01	10.54	13.5	167	45.90	8.08	**3.36**	3.11
330	51.67	11.04	14.0	104	49.60	9.09	1.74	2.07
340	52.92	10.20	14.5	29	50.30	9.18	1.34	2.62
2750				1114				

Remark: In the „t" column the thickly set values indicate the differences provable by 95%, or by higher probability.

Table 4. Body weight parameters of the girls of Szeged

1981/82			Age	1966/67			t	Diff.
n_1	$\bar{x}_1$	s_1		n_2	$\bar{x}_2$	s_2		
38	35.44	6.71	10.0	58	33.10	5.43	1.88	2.34
395	35.70	7.75	10.5	69	34.20	6.81	1.51	1.50
487	37.53	8.82	11.0	72	36.60	8.52	0.84	0.93
480	39.68	8.71	11.5	113	38.50	7.23	1.34	1.18
473	42.97	9.28	12.0	119	40.00	7.23	**3,25**	2.97
474	45.80	9.72	12.5	112	44.10	8.83	1.69	1.70
528	47.29	9.72	13.0	120	46.30	8.81	1.02	0.99
466	50.43	11.06	13.5	89	49.00	8.58	1.15	1.43
392	51.84	8.89	14.0	57	49.80	8.24	1.63	2.04
377	53.12	9.68	14.5	23	51.90	12.07	0.58	1.22
4110				832				

Remark: In the „t" column the thickly set values indicate the differences provable by 95%, or by higher probability.

The average values of the normal breast circumference in several age groups in the case of boys were lower in 1981/82 than in 1966/67 (10.5—11.5; 12.5; 14 years of age) but these differences were not provable by t-test (Table 5). In the case of girls the

Table 5. Normal chest circumference parameters of the boys of Szeged

1981/82			Age	1966/67			t	Diff.
n_1	$\bar{x}_1$	s_1		n_2	$\bar{x}_2$	s_2		
26	66.39	5.62	10.0	70	66.00	4.18	0.37	0.39
251	67.31	5.83	10.5	68	67.50	4.59	0.35	—0.19
289	68.53	6.22	11.0	71	68.70	4.53	0.22	—0.17
303	69.54	6.60	11.5	122	70.60	5.72	1.55	—1.06
286	71.39	6.75	12.0	150	70.80	5.56	0.92	0.59
336	72.71	7.34	12.5	182	73.00	5.69	0.46	—0.29
268	74.67	6.51	13.0	151	74.10	5.62	0.90	0.57
320	76.61	7.56	13.5	167	76.30	5.54	0.47	0.31
330	78.68	7.52	14.0	104	78.80	6.51	0.15	—0.12
340	80.49	6.94	14.5	29	79.40	6.22	0.82	1.09
2749				1114				

averages of the latest sample — with the exceptance of the 10 years old girls — were lower in every other age group in 1981/82 than those of the 15 years earlier samples, one part of which (11—11.5 and 13 years old girls) was also provable by statistic methods (Table 6).

Table 6. Normal chest circumference parameters of the girls of Szeged

1981/82			Age	1966/67			t	Diff.
n_1	x_1	s_1		n_2	x_2	s_2		
38	66.84	5.58	10.0	58	66.60	4.55	0.23	0.24
395	67.33	7.24	10.5	69	68.40	6.54	1.15	—1.07
487	68.72	7.78	11.0	72	71.10	6.31	**2.55**	—2.42
480	70.63	7.75	11.5	113	72.40	6.67	**2.23**	—1.77
473	73.59	8.05	12.0	119	74.20	6.04	0.77	—0.61
474	76.38	8.28	12.5	112	77.60	7.13	1.44	—0.22
528	77.94	8.31	13.0	120	80.20	7.50	**2.74**	—2.26
466	80.40	9.20	13.5	89	81.20	6.55	0.78	—0.80
392	81.66	7.34	14.0	57	82.10	5.87	0.43	—0.74
377	83.30	7.99	14.5	23	84.20	8.60	0.52	—0.90
4110				832				

Remark: In the „t" column the thickly set values indicate the differences provable by 95%, or by higher probability.

It should be mentioned that all measurements were made by the author, therefore the difference cannot be considered as menthodological differences.

From the above, therefore, the interesting experience can be concluded on the basis of the measurings carried out at various periods that while between the years 1958/59 and 1966/67 the acceleration process could be proved unanimously, between the years of 1966/67 and 1981/82 this only limited to the body weight and height, which, however, cannot be observed in every age group. The normal breast circumference — which is one of the indicatives of the size of the chest and also the vital capacity of the lung — showed a lag, in case of both sexes, compared to the averages of the earlier 15 years. Although this cannot be proved statistically in the case of the boys, it is a factual data. This latter observation calls attention to the fact that the motion developing the respiration of adolescent school children is not ensured as should necessary (firstly due to lack of time), which explains to a certain extent the increased fatigability of these children.

We should only like to mention as an interesting fact that similar experience was gained when comparing the body measurement averages of children of nursery age (FARKAS, 1983).

We do not wish to report in detail on the degree and causes of the menarche median changes, since this will be the subject of another study. However, we should like to mention that in the last decades we obtained the following values from the examination of girls of Szeged (FARKAS, 1964, 1972):

In 1958/59 the median on the basis of the data from 1441 girls was 13.20 years,
In 1961 the median on the basis of the data from 1469 girls was 13.03 years,
In 1966/67 the median on the basis of the data from 1136 girls was 12.73 years,
In 1981/82 the median on the basis of the data from 6984 girls was 12.77 years.

It can be seen from the comparison that the period of puberty between 1958/59 and 1966/67 definitely decreased, while it showed no essential changes during the last 15 years.

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Address of the author:
DR. GY. FARKAS
Department of Anthropology
A. J. University, H-6701 Szeged
P.O. Box 660, Hungary

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APPEARANCE AND INCIDENCE OF CORONARY DENS INVAGINATUS ON THE BASIS OF STUDIES ON RECENT AND PALEOANTHROPOLOGICAL SAMPLES

G. KOCSIS and ANTÓNIA MARCSIK

Clinic of Dental and Oral Surgery, Medical University of Szeged, Department of Anthropology, Attila József University, Szeged
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Abstract

With the help of x-ray pictures authors searched for tooth enamel invaginations of the dilated from on 500 and 277 upper lateral incisors, resp., studying recent and paleoanthropological samples. The frequency of appearance of the invagination was determined: 2.4% in recent samples, and 7.2% in paleoanthropological ones. The found values do not differ greatly from those reported in the literature. Authors also studied the frequency of bilateral appearance, the multiple occurrence, variations between the two sexes, as well as the medical historical aspects of the phenomenon. A summary is given of the histology and etiology of the invagination, on the basis of literary data. Such a study has not been carried out so far in the Hungarian paleoanthropological series.
Key words: invagination, foramen coecum, recent and paleoanthropological samples.

Introduction

The infolding of the surface layer of an organ into the organ itself is a known clinical picture called invagination. Intestinal invagination is frequent, when the oral intestine part infolds into the aboral section of the intestine. In case of teeth, the phenomenon is called invagination when the surface layer turns towards the inside of the tooth. If the opening of the invagination is on the crown of the tooth, we speak of coronary invagination. When the starting point is the surface of the root, this is called the rooted form. Regarding it's expansion the coronary invagination can be divided into the surface and deep form. The difference is decided by whether the invagination overreaches the enamel-cement border or not. Certain authors, like HALLETT (1953) and SCHULZE (1970) hold the foramen coecum as an invagination, too, while others only hold this to be a coronary variant. The process may start from the foramen coecum or from a corner; according to observations this latter results an invagination of extreme size.

The disease has been known for over 100 years. HUNTER (1951) studied the question of priority; according to him it was first reported by SALTER in 1855, SOCRATES in 1856, and TOMES in 1859 in his work „A System of Dental Surgery". According to SCHULZE (1970) however, the case described by SALTER was with all probability a geminate tooth. We have found an illustration of a tooth in the first book written in Hungarian, dealing with dentistry (BARNA, 1871). According to our opinion this is an invaginated left upper molar tooth (Fig. 1).

The alteration was given many different namings. The naming „dens invaginatus" given by HALLETT in 1953 is also in accordance with the conception of the coronary and rooted alteration. The naming dens in dente (BUSCH, 1897) used earlier is inaccurate in its original meaning, but has become so inveterate that even the WHO (1978) mentions it besides the previous one. Nevertheless, it can only be used in such form

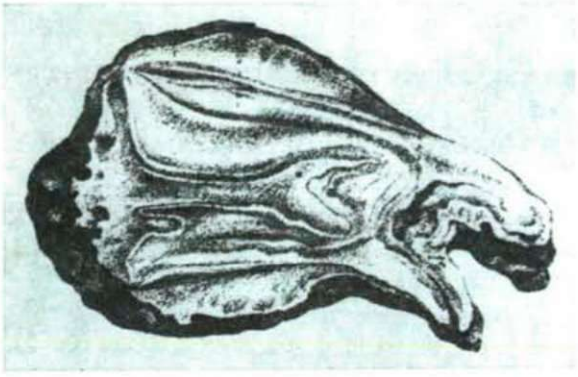

Fig. 1. Illustration of the left upper molar tooth from the book of BARNA published in 1871. The cross sectional picture (right side) shows invagination.

of the coronary type where the tissue elements of the invagination are enamel and dentin, furthermore, where the invaginated part is so large that the part of the tooth which has inverse structure fills out completely the pulp cavity of the original tooth structure, deforming the tooth.

Materials and Methods

1. The x-ray pictures of 500 upper small incisors were studied, obtained from the patient material of the Department of Orthodoncy, Clinic of Dental and Oral Surgery, Medical University of Szeged.

2. Further specimens for studying were obtained from skulls from three cemeteries of the Avarage, originating from the 7.—8. century (Szeged-Kundomb, Szeged-Makkoserdő, and Szeged-Fehértó). Totally 277 upper small incisors were studied from 5510 teeth of 225 skulls (120 males and 105 females).

The ADI value of the small incisors was 0.43 (thus 43% was missing from the theoretically 450 pieces of upper small incisors). X-ray pictures were taken where foramen coecum was observed.

In both experimental groups the frequency of dilated invaginations was determined, which according to our opinion corresponds to the 3rd type according to the classification of HALLETT (1953) (Fig. 2), furthermore we looked for sexual dimorphism.

By evaluating the x-ray pictures, in the majority of the cases the abnormality could be determined, this is why we used this method. The result of the x-ray is uncertain in the cases of rather insignificant, or extremely large invaginations, resp. The invaginations may also be mimicked by projections, geminate forms and odontomas.

The received data were compared with the similar data found in the literature both in the case of the recent and paleoanthropological findings.

Results and discussion

Recent samples

From the 500 x-ray pictures of the upper second incisors, invagination of the dilated form was found in 12 cases, meaning a frequency of 2.4%.

From the 12 invaginated teeth three pairs were of bilateral appearance (6 invaginated small incisors), in 4 cases alterations were only observed on one side, in the right upper double teeth. In 2 cases only the x-ray picture of the small incisor on one side could be evaluated, thus in the case of the symmetry studies 10 instead of the 12 invaginations were examined. Accordingly, the unilateral-bilateral appearance had a frequency of 57—43% in our material.

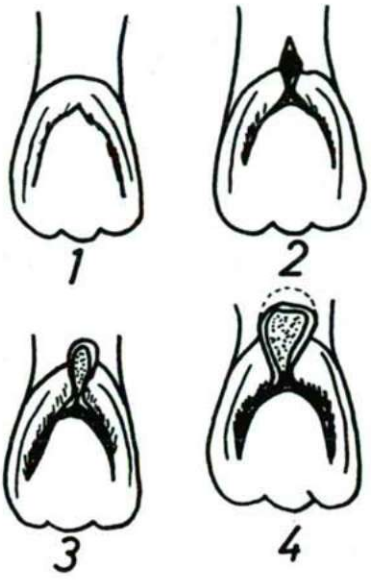

Fig. 2. The four types of invagination according to the expansion:
1. cleft palate enamel edge
2. deep foramen coecum; non-dilated invagination
3. dilated invagination
4. deep invagination; dens in dente (HALLETT, 1953)

Fig. 3. Geminated invagination in two supernumerary teeth (mesiodens).

In one case, as a secondary finding, we observed invagination in a supernumerary tooth, two-two enamel indentations in double mesiodenses (Fig. 3).

This alteration was found to be three-times more frequent in the case of females than in males.

Paleoanthropological samples

225 (120 male and 105 female) skulls were studied; from which a total of 20 invaginations of the small incisors (Fig. 4a, 4b) were found in 11 cases (4.89%). 5 were male, and 6 were female skulls.

In the 225 skulls 277 upper small incisors were present, 155 incisors belonged to male and 122 to female skulls. During the course of our studies we also searched for foramen coecum. Such abnormality was found in a total of 52 small incisors (18.7%), 25 on males, 27 on females teeth. From these 19 pairs (38 pieces) had symmetric appearance and one foramen coecum was unilateral. In 13 cases only the small incisor on one side was present.

On the x-ray pictures prepared of the foramen coecum 20 dilated invaginations were found on 10—10 male and female teeth, respectively. This means a frequency of 7.2%. Invagination was found on both-sided small incisors in 9 cases (18 pieces), while no unilateral appearance was observable. In two cases only one of the small incisors could be examined, therefore, these were not taken into consideration when studying the symmetrical appearance. On the basis of this the bilateral appearance could be regarded almost 100% in the paleoanthropological findings.

The literary data on the prevalence of invaginations also differ within a population. This is explained partly by the fact that the studies are not uniform, since certain authors (VÉGH, 1974; GOTOH et al. 1979) only studied the upper small incisors, while others also involved in their studies the whole row of teeth, including the probable supernumerary teeth, too. As we have already mentioned, several authors hold the foramen coecum, as well as the cleft palate enamel edge as a form of invagination, too, while AMOS (1955) only studied the appearance of the superficial-type invagination.

Concerning the various types of teeth the abnormality is the most frequent in the upper second incisors, this is why many authors — including us — only study these teeth. According to HALLETT (1953) invagination is 8 times more frequent in the upper double teeth than in the upper single teeth.

Invagination frequently occurs in the supernumerary teeth. Compared to these, the incidence is rare in other types of teeth. SCHAEFER (1953) elaborated the data of over 100 authors, according to which this abnormality may occur in every type of teeth. Recently BANNER (1978) reported on invagination on four lower premolars, and CONKLIN (1978) on two lower incisor teeth. From the Hungarian authors BRUSZT (1950), MIKLÓS (1976) and VERECKEI (1977) described cases of deep invagination in upper lateral incisors, VÉGH (1974) reported on poor and deep invaginations, ADLER (1939) gave a report on such an abnormality in a lower incisor, while KÉRI and BAKODY (1973) as well as VÉGH (1978) in the case of the lower canine tooth. In our opinion the afore—mentioned illustration of BARNA (1871) also shows this abnormality on an upper molar.

The data concerning the prevalence of coronary invagination are summarized in Tables 1 and 2. It can be seen from these two Tables that the frequency regarding the upper small incisors is a value around 4%, TWIESSELMANN and BRABANT (1967) did not find any invaginations, and according to the studies of HALLETT (1953) the prevalence of invagination in concern of the upper small incisors is a value of 48.98%; this

latter, however, includes all four types (Fig. 2), from which the 3rd type of invagination is a value of 4.44%.

The prevalence values found by us (2.4% in negative direction in the recent samples, while 7.2% in positive direction in the paleoanthropologic samples) slightly differ from those of the literary data, nevertheless, the 4.89% skull number average of the paleoanthropological samples fits into the average value determined in the case of the recent group, and on the other hand, it also does not differ essentially from the 4.2% value of BRABANT and SAHLY (1962) found in their material from Neolithic.

In the case of the paleoanthropological findings the 18.7% frequency of the foramen coecum (also including the invaginated teeth) is approximately identical with the appearance of the 2nd and 3rd type of invagination according to HALLETT (1953). (In his report the lst and 2nd type of invagination of the upper small incisors appear in 43.17% referring to individuals, and the 3rd type appears in 4.61%. The tooth average is 25.77% in the case of the lst type, 16.55% in the case of the 2nd type, which corresponds the best to the foramen coecum; and the 2nd and 3rd types together are 20.99%.

On the basis of the afore-mentioned, therefore, we think that the difference from the literary data is not considerable, but can rather be characterized as extreme data. Nevertheless, the fact that the studies carried out with same procedure resulted a prevalence value of 2.4% in case of the living group, while a value of 7.2% was obtained in the group that lived 1200 years ago, encourage us to continue our researches in this direction.

Table 1. Frequency of coronary invagination and its distribution according to sexes (Data related to living population)

Authors	No. of studied persons or teeth	Occurrence %	Males %	Females %
SHAFER, 1953	2452 persons	1.26	58.0	42.0
AMOS, 1955	1000 persons	5.1	49.0	51.0
AMOS, 1955	203 persons	6.89	—	—
GOTOH et al. 1979	766 persons	2.6	—	—
HALLETT, 1953	1172 upper small incisors	4.44 3rd type invagination	—	—
STEPHENS, 1953	300 upper small incisors	5.0	—	—
VÉGH, 1974	500 upper small incisors	3.6	—	—
Present study	500 upper small incisors	2.4	25.0	74.0

Table 2. Frequency of coronary invagination and its distribution according to sexes. (Data related to paleoanthropological series)

Authors	No. of studied skulls or teeth	Occurrence %	Males %	Females %
BRABANT and SAHLY, 1962	3008 teeth late neolitic	4.2	—	—
TWIESSELMANN and BRABANT, 1967	605 skulls IV.—X. century	0	—	—
Present study	225 skulls	4.89	45.2	54.8
Present study	277 upper incisors, resp. VII.—VIII. century	7.2	47.6	52.4

Sexual differences in the recent sample were not demonstrable on the basis of summarizing the data found in the literature. Significant differences between sexes were also unobservable in the case of the series from the 7.—8. century, both in regard of the appearance of invagination and foramen coecum.

The alteration may appear at the same time on several teeth within a mouth. SWANSON and MCCARTHY (1947) were the first to report on the bilateral form. According to VÉGH (1974) this is not rare; from 18 cases 10 were bilateral (5 pairs) and 8 were unilateral. According to AMOS (1955) the frequency of the bilateral, non-dilated invagination is 40%. According to our studies carried out on the living population the bilateral occurrence is 43%. In the paleoanthropological samples the bilateral appearance of the foramen coecum is 19-folds that of the unilateral occurrence, and the invagination was found to be of symmetrical appearance in 100%. This result is near that of HALLETT (1953), who during the study of 586 canine teeth found invagination of symmetrical appearance in 278 cases (types 1—4) and observed unilateral occurence only in 13 cases (95.22% and 4.48%, respectively).

The multiple occurrence of dens invaginatus has also been described in the case of a single tooth. CONKLIN (1975) reported on a case of bilateral double invagination, MANSON-HING (1960) described a case of double invagination appearing on all the four premolars, MADER (1977) published the case of triple appearance, TOWNEND (1975) reported on multiple invagination in the upper small incisor, in the area of which the tooth became fungous in structure. In their paper, GUSTAFSON and SUND-

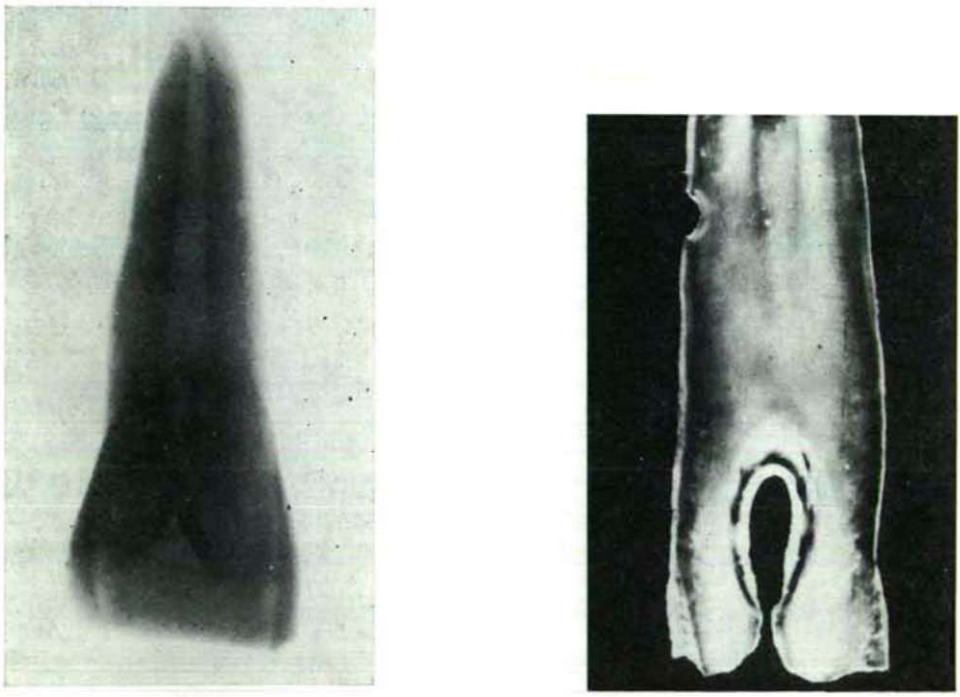

Fig. 4 a) X-ray picture of the right upper double tooth from the Avar-age (inventory no. 1562, tomb no. 323, Fehértó-A.). Invagination of dilated form can be seen in it.
b) The polished picture of the previous tooth, with the magnification of the invagination. The enamel covering the surface of the invagination has an amorphous structure, it became poorly calcified.

BERG (1950) mention MILLER (1901), who described a tooth which became invaginated in 15 different places (although this is not a typical dens in dente in our opinion). Multiple invaginations are not rare in the supernumerary teeth, which fact is also supported by the case observed by us (Fig. 3).

The alteration may also be accompanied by other abnormalities. The presence of invagination is rendered probable by the unnormal shape of the crown of the tooth, golf-club or barrel shape, the enlargement of the lingual corner (talontip) on the front teeth (OEHLERS, 1957, SCHULZE, 1970, GOTOH et al. 1979). It is doubtless that the two characteristics — the invagination and the divergency in shape — may accompany each other, nevetheless, the joint occurrence is not exclusive.

MADER (1979) observed gemination and double invagination on one and the same tooth. SCHULZE (1972) also observed it together with the formation of a geminated tooth (regarding the invagination developed in such a way as another form), and the invagination of a lower canine tooth reported by VÉGH (1978) is also a similar case. The case of SHIFMAN and TAMIR (1979) is the concretion of an invaginated upper small incisor and a supernumerary tooth. WESTBORG and JULIN (1974) found macrodontia, multituberculism and central cusps apart from pulp invaginations. CASAMASSIMO et al. (1978) described such a case where besides dens invaginatus, microdontia and taurodontia were also observed.

Histologically the enamel of the invagination always developed and became calcified poorly (Figs. 4a and 4b). HAMMER (1935), KÉRI and BAKODY (1973) and other authors only found irregular masses. According to FISCHER (1936) the dentin tubules are also irregular. Several authors, like BUSCH (1896), FISCHER (1936), GUSTAFSON and SUNDBERG (1950), BRUSZT (1950) found connection between the pulp of the „dens in dente" type of invagination and the pulp ventricle. Many authors hold this as an explanation for the fact that these teeth lose their vitality soon. However, TAYLOR and MCDANIEL (1977) reported on two cases in which the pulp of the invagination was independent of the dental pulp, communicating parallel with it. In one of the cases the tooth appeared to be live on the effect of electric stimulus, but on the x-ray picture a granuloma was observable on the side of the bone of the invaginated pulp. The tooth was necrotic in the other case. Studying the small invagination starting from the foramen coecum, KRAMER (1953) did not find any correlations between the previously described two pulps.

The etiology of the abnormality is a question still much debated nowadays. In the case of the coronary from there are two conceptions; in the wording of SCHULZE (1970) the theory of one tooth and the theory of two teeth, respectively. The accepted form of the two teeth theory was elaborated by BRUSZT (1950). In his opinion two tooth embryos (one is usually a supernumerary tooth) become geminated due to pressure, but the enamel remains in a small part between them and this gives the invagination. The notion is found to be supported by the afore-mentioned connection between the invaginated space and the pulp ventricle.

Several conceptions arose regarding the invagination originating from one tooth. These were summarized by HALLETT (1953), while from the Hungarian authors it was MIKLÓS (1976) who dealt with these theories. Accordingly, the cause may be proliferation growth pressure, growth lag, infection, atavism, trauma, and even genetic factors may play role. The namings odontoma (HUNTER, 1951; OEHLERS, 1957), hamartoma, hamartoblastoma (TRATMAN, 1951) which assume tumorous origin are incorrect. Even if we follow the conception of HUNTER (1951), RUSHTON (1958) and others who speak of tumorous proliferation, this can only be accepted as by OEHLERS (1957), according to which author there may also be invaginations of proliferative origin. This tumorous proliferation, however, is only an assumption, needing further studying.

We should accept the classification of PONGRÁCZ (1965) who sharply separates the dens invaginatus from the odontomas.

On the basis of the literary data and our own studies, our opinion is that considering the tissue structure of the tooth invaginations and the alterations of the tooth and dental pulp, there are two kinds of abnormalities. One form — which we also studied in this work — in the alteration remaining from the foramen coecum, the true invagination of the tooth, the etiologic factors of which were listed when discussing the one tooth type. The other form — which may be in connection with the geminative abnormality — is the anomaly, more or less distorting the tooth and causing the necrosis of the dental pulp in a large percentage. The true etiology of this type has been described by BRUSZT (1950), SCHULZE (1972) and VÉGH (1978).

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Adress of the authors
DR. G. KOCSIS
Clinic of Dental and Oral Surgery,
Medical University of Szeged
6720 *Szeged*, Lenin krt. 64. Hungary
DR. ANTÓNIA MARCSIK
Department of Anthropology,
Attila József University 6701
Szeged, Hungary P.O. Box 660,

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SHORT COMMUNICATIONS

ENDOINFUNDIBULAPOLLIS DISTINCTUS R. TSCHUDY 1975, FROM THE UPPER CRETACEOUS FROM THE SOUTHERN PART OF HUNGARY. FIRST OCCURRENCE OF THIS FORM-GENUS FROM EUROPE

M. KEDVES

Department of Botany, A. J. University, Szeged
(Received July 31, 1982)

The Senonian sediments of the bore-hole of Csávoly-I (South Hungary) were investigated palynologically. The spore-pollen composition is different from those described from the Bakony Region (Transdanubia). The presence of *Endoinfundibulapollis distinctus* R. TSCHUDY 1975 is also a pecularity of this assemblage, because this form-genus has been reported previously only from the Atlantic Coastal Plain of North America. It was belived that this is a North American pollen type. DR. R. TSCHUDY (United States Department of the Interior, Geological Survey, Denver, Colorado, USA) checked my determination and in a letter of April 2, 1981 he wrote the following: "From your pictures I am confident that you have found the same species from the southern part of Hungary that we have in eastern North America. In eastern North America this species is found in the Santonian, Campanian, and early Maestrichtian. I have not found it in the Aquilapollenites province."

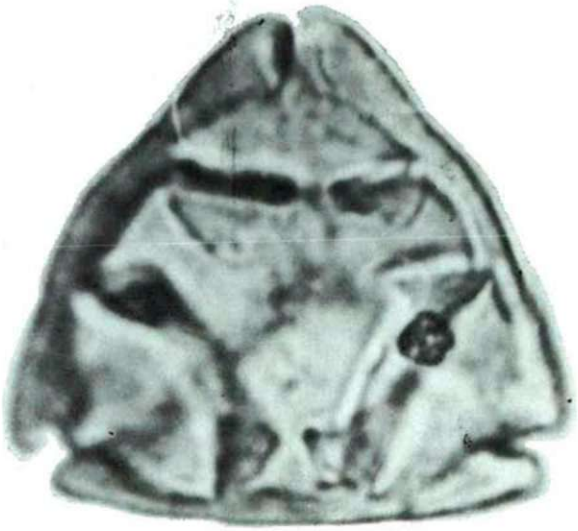

Fig. 1. *Endoinfundibulapollis distinctus* R. TSCHUDY 1975, slide: Csáv-I-2-6; 17.6/111. 7, x 3000.

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Address of the author:
DR. M. KEDVES
Department of Botany A. J. University
H-6701 Szeged, P.O. Box 657, Hungary

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THE DEPENDENCE OF LIGHT-INDUCED VIOLAXANTHIN TRANSFORMATION ON THE RATIO OF STROMA LAMELLAE

I. MARÓTI and SZERÉN PATAKY

Department of Botany, Attila József University, Szeged
(Received November 1, 1982)

In our earlier publications (MARÓTI, *1976;* MARÓTI and GÁBOR, 1976) it was assumed that there is firstly cyclic electron transport in the stroma lamellae, and this is independent of the linear ($H_2O \rightarrow NADP^+$) electron flow found in the grana. It is known that in the inductional phase photosynthesis the linear electron transport hardly functions (WALKER, 1976). The light-induced acidification of the intrathylakoid space (optimal: pH 5) activates the de-epoxidase enzyme (HAGER and PERZ, 1970), therefore the decrease in violaxanthin is the endogeneous indicator of the temporal accumulation of protons (SIEFERMANN—HARMS et al., 1980). In the loculus the protons are even capable of accumulation (due to the cyclic electron transport) when the linear electrontransport is hindered (SIEFERMANN—HARMS et al., 1980; CROWTHER and HIND, 1980). On the basis of the afore-mentioned it is expectable that the amount of violaxanthin transformed in the inductional phase would be proportional to the area of the stroma lamellae.

For our experiments such inbred corn lines were used: *Zea mays* L., *Pioneer* 165 and 523, which significantly differ in their mesophyll chloroplasts even in long-day light (light-dark periods, LDP, of 16—8 hours), and in short light-dark periods the ratio of stacked and single lamellae varies diversely (MARÓTI et al., 1982).

The de-epoxidation of violaxanthin developing on the effect of strong light of 1, 2 and 4 min. duration (400—900 Wm^{-2}) was studied with discs taken from the 4th leaf of 5 weeks old plants. The pigments were extracted, separated and measured (MARÓTI and GABNAI, 1971). To determine the ratio of the stroma lamellae and partition cc. 30 chloroplast membranes — selected according to types and treatment with (—) light — were measured on electronmicroscopic pictures.

The amount of violaxanthin transformed on the effect of strong light shows tight relationship with the ratio of the stroma lamellae in the first and second min. (Table 1).

Table 1. Decrease of violaxanthin (μg/mg chlorophyll a) in the 4th leaf of 5 weeks old corns on the effect of 2 min. long (800 Wm^{-2}) strong light. The ratio of stroma lamellae before de-epoxidase experiments developed on the effect of 16—8 hours and 30—15 min. long LDP-s and 32 Wm^{-2} light intensity, resp.

Corns were grown in light-dark periods	Conversion of violaxantin (μg/mg chlorophyll a/2 min')		% of stroma lamellae	
	P 165	P 523	P 165	P 523
16—8 h	6	17	30	48
30—15 min	20	11	46	35

In the leaves of plants utilizing well the short period of light (MARÓTI, 1981) — in the inductional phase of photosynthesis — the de-epoxidation of violaxanthin develops faster than in those leaves of plants on the development of which the short LDP is unfavourable.

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Address of the authors:
DR. J. MARÓTI
DR. SZ. PATAKY
Department of Botany, A. J. University
H-6701 Szeged, P.O. Box 657, Hungary

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NEW DATA OF BETHYLIDAE FROM THE PRIESNER'S COLLECTION (HYMENOPTERA)

L. MÓCZÁR

Department of Zoology, Attila József University, Szeged
(Received September 15, 1982)

Abstract

Two new males of *Anaylax aegyptius* MÓCZÁR, 1978 and *Metrionotus egypticus* MÓCZÁR, 1974, as well as, new localities for *Mesitius spathulifer* PICARD, 1932, *M. apterus* CAMERON, 1888 and *M. ghilianii* SPINOLA, 1851 are published.

Anaylax aegyptius MÓCZÁR, 1978

Specimens examined: 2 ♀ Meadi, Egypt 9.5.33 and 26.6.33 (Detritus) leg. DR. H. PRIESNER; 1 ♂ *nov.* "Pyramids Egypt 9.11.33 DR. H. PRIESNER", "H. Priesner Collection 1969" (USNM Washington).

♂. — Length 1.7 mm. Similar to female, light yellowish brown, only flaggelar joints 4—13 and coxae brown, abdomen largely reddish brown translucent. Wings normal, antenna with long suberect hairs, latter hardly shorter than width of joints.

Head only slightly longer than broad (25:21, at magnification x 50) remarkably broadened behind eyes (this breadth equaling larger diameter of eye, 9:9); surface of head alutaceous, more shining than in female; ocelli in a sharp angle, POL:OOL = 3:6, outer margins of ocelli with fine grooves; eye separated from mandibles by half distance of its length (4.5:9); antenna slender, all joints at least twice as long as broad, and with parallel sides excepting joints 1—2, joint 1 slightly curved, lower part of 2 convex, length (and breadth) of antennal joints 1: 2: 3—10: 11—12: 13 = = 8(3): 6(2.5): 5—5(2): 4.5(2): 6(2). Pronotum slightly broader in front than its length medially (without collar) (12:10), longitudinal furrow not present, surface smooth, shining, finely alutaceous together with mesonotum and scutellum. Mesonotal furrow not present. Propodeum remarkably long, distinctly longer than its half diameter posteriorly (10:7.5), lateral spines very short, hardly distinct, all carinae present, areas finely sculptured, shining. Abdomen smooth, polished only with very few fine punctures.

Distribution. Egipt (MÓCZÁR, 1978). Only the holotype was known.

Mesitius spathulifer PICARD, 1932

Specimen examined: 1 ♀ Gerusalemme 12.3.33 leg. SATZMAYR.

Distribution. Syria, Jordan (MÓCZÁR, 1970), Palestine.

Mesitius apterus CAMERON, 1888

Specimen examined: 1 ♀ Gerusalemme, 11.3.33 leg. SATZMAYR.

This specimen differs from the typical material (MÓCZÁR, 1970) by its head being partly dark red translucent, by the red propodeum (not blackish), by the lateral area of propodeum being more convex, by the pronotum with denser minute punctures and consequently hardly shining.

Distribution. Gibraltar, Morocco (MÓCZÁR, 1970), Palestine.

Mesitius ghilianii SPINOLA, 1851

Specimen examined: 1 ♀ Egypte, Abausir 19.4.35 leg. RABINOVITCH.

This very small (3.6 mm) specimen differs from the larger (4.8 mm) specimens by the very small grooves along the lateral sides of ocelli.

Distribution. Tunis, Gibraltar, Sicily, Corfu (MÓCZÁR, 1970), Egypt.

Metrionotus egypticus MÓCZÁR, 1974

Specimens examined: 1 ♀ Helwan 11.2.36 Coll. FARAG; 1 ♂ *nov.* "Fayoum Egypt 13.9.29 DR. H. PRIESNER", "H. Priesner Collection 1969" (USNM Washington).

♂. — Length 3.4 mm. Similar to female, head and thorax red, mandibles, tegulae more yellowish, antenna also red, only last 2—3 joints brownish, legs partly yellowish red, abdomen black, segment 1 and last segments brownish red translucent. Wings, body hairs as in female, antenna with white suberect hairs, latter only half as long as width of joints.

Head rounded, finely granulated between punctures, frontal sulcus indistinct, ocelli in an acute angle, POL:OOL = 7:8 (not 5:9 ♀), groove distinct along ocelli, malar space as in female. Antenna slender, all joints distinctly longer than broad, length (and breadth) of joints: 1: 2—3: 4—7: 8—11: 12: 13 = 12(6): 9—9(4.5): 8—8 (4): 7—7 (4): 6(3): 9(2.5). Pronotum rather broad nearly one-third broader in front than long medially (without collar) (27:20), lateral side slightly concav owing to a wrinkle emerging on lateral side and reaching the corners, surface granulated and shallowly punctured, less shining than in female, longitudinal furrow narrow and deep. Mesonotum, scutellum granulated, matt, mesonotal furrow not present. Propodeum similar to that of female only lateral spine distinctly shorter, less than half length of propodeum medially (8:18). Abdominal tergite 1 polished, 2 broadly alutaceous basally and with distinct, scattered punctures medially and posteriorly.

Distribution. Egypt (MÓCZÁR, 1974) (only 2 ♀ were known).

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Address of the author:
PROF. DR. L. MÓCZÁR
1117 Budapest
Mészöly u. 6.
Hungary

INFORMATIONS

HUNGARIAN-POLISH SYMPOSIUM: EFFECT OF PHYSICAL AND CHEMICAL AGENTS ON BIOMOLECULES, SZEGED, 9—13 JUNE, 1981

The Department of Biophysics and the Department of Biochemistry at the University in Szeged maintain direct connections with the Institute of Biochemistry and Biophysics of the University of Lodz on the basis of the contract of friendship between the two Universities, Within the framework of this connection symposia are held alternately in Lodz and in Szeged at two-yearly intervals. The latest symposium was organized by the Szeged Departments. The following lectures were delivered at the meeting:

LEYKO, W.: Antioxidative defense enzymes in fish.

GONDKO, R.: Hemocyanin of fresh water crayfish.

SIMON, M.: Studies of the effect of paraquat on liver microsomal enzymes in carp.

NEMCSÓK, J. and BOROSS, L.: Studies of the physical and chemical treatments on the blood enzymes of various fish.

DÉR, A.: Studies on the fish transaminase isoenzymes by polyacrylamide gel electrophoresis.

GULYÁS, M.: Effect of immobilization on the heat stability of some oxidases.

JÓNÁS, E. and BOROSS, L.: Preparation and properties of immobilized JB-l protease.

KRAJEWSKI, T.: Resemblance of terminal plasmin degration products of mammalian and avian fibrinogen.

GRABOWSKI, J., LESZCZYNSKA, and NOWAK, A.: Biomass determination of viable microorganisms by the fluoro-pigment method.

MATKOVICS, B., VARGA, SZ. I., SZABÓ, L., BARABÁS, K. and BERENCSI, G.: The present status and future plan in our peroxide metabolism enzymes studies.

FRACKOWIAK, D., STILLMANN, M., BAUMAN, D. and MANIKOWSKI, H.: CD and MDC spectra of chlorophylls in nematic liquid crystals.

HERCZEG, T., LACZKÓ, G., MARÓTI, P. and SZALAY, L.: Microsecond delayed fluorescence of the second photochemical system of photosynthesis.

VOZÁRY, E.: Polarization properties of modified purple membranes.

JADZYN, C. and FRACKOWIAK, D.: Photovoltaic effects of flavins.

BÖDDI, B. and LÁNG, F.: Aggregation and spectroscopic properties of protochlorophyll in detergent micelles.

SZITÓ, T., HEVESI, J. and BÁLINT, E.: Connection between the stucture of the micellar system and the energy migration.

SZIGETI, Z.: Enhancement of chlorophyll photooxidation with benzonitrilite type herbicides and protection with some reducing agents.

LASKAY, G., FARKAS, T., LEHOCZKI, E. and SZALAY, L.: Manipulation of chloroplast lipids and fatty acids by pyridazinone herbicides.

GARAB, Gy., ZIMÁNYI, L. and FALUDI-DÁNIEL, Á.: Interpretation of the complex kinetics of the absorbance change A515 in chloroplasts.

DUDA, W.: Effect of gamma-irradiation on the primary structure of bovine hemoglobin.

WALTER, Z.: Effect of malathion on genetic material of human lymphocytes.

TARNÓY, K.: Fluorescent labeling of bacteriophages.

TÓTH, K., PATAKI, K. and ASLANIAN, D.: CD-melting monitored conformational changes on UV irradiated T7 bacteriophage.

FEKETE, A., RONTÓ, Gy., TARJÁN, I. and SUGÁR, I.: Kinetics of UV-photodamage of T7 and MS2 bacteriophages at higher doses.

GRABOWSKI, J.: Remarks on the possibility of the remote sensing of the biomass production in the world ocean.

PUTNOKY, P. and BEREK, I.: Genetic mapping in *Rhizobium meliloti* 4.

OLASZ, F., DORGAI, L., BERÉNYI, M., DALLMANN, G., PÁY, A. and OROSZ, L.: Analysis of cystein transducing 16—3 phages of *Rhizobium meliloti*.

DORGAI, I., DALLMANN, G., OLASZ, F., PÁY, A. OROSZ, L.: Analysis of delation and inversion mutants of *Rhizobium meliloti* temperature phage 16—3 and orientation of genetic and physical map.

DUDÁS, B., ERDEI, S., DUDA, E. and OROSZ, L.: Identification of structural proteins of *Rhizobium meliloti* temperature phages 16—13.

L. SZALAY

FOURTH INTERNATIONAL LUMINESCENCE CONFERENCE, SZEGED, 24—27 AUGUST, 1982

This Conference is organized at three-yearly intervals; separate sections deal with the biological applications of luminescence. This year the following lectures were delivered on this subject:

LEUPOLD, D., WIESNER, B., EHLERT, J., MORY, S. and VOIGT, B.: Properties of excited states of chlorophyll-a in solution.

PASCHENKO, V. Z.: Pulse fluorometry of energy migration processes in higher plant chloroplasts.

KONONENKO, A. A., KNOX, P. P., VENEDIKTOV, P. S., GARAB, Gy. J. and FALUDI-DÁNIEL, A.: Electric polarization and thermoluminescence of chloroplasts.

NÉMET, B., VARGA, É. and KOZMA, L.: Quantitative analysis of very small amounts of biological and chemical materials with pulsed laser fluorimeter.

VASS, I.: Connection of thermoluminescence and phase transitions in chloroplasts.

DEMETER, S. and VASS, I.: Isokinetic relationship between the activation energy and entropy in the thermoluminescence of herbicide-treated chloroplasts.

MENDE, D., MARÓTI, P. and WIESSNER, W.: Comparative studies on fast cytochrome reaction in microalgae induced by laser excitation.

DRABENT, R., LACZKÓ, G., MIELOSZYK, J., SIÓDMIAK, J. and BYSTRA, K.: The fluorescent complex formed by flavomononucleotyde in polymer matrix.

GRABOWSKI, J. and ZILINSKAS, B.: The determination of absorption spectra of two kinds of chromophoric groups in phycoerythrin aggregates with the use of measured polarized fluorescence excitation spectra.

VACEK, K. and PANCOSKA, P.: Fluorescence study of pigment-protein interactions in photosynthesis.

KOWALCZKY, A. A., KNUTSON, R., BARKLEY, M. D., CHRISTY, R. and BRAND, L.: Anisotropic rotations of perylene in liposomes.

GANAGO, A. O., GARAB, Gy. and FALUDI-DÁNIEL, A.: Polarized luminescence of chloroplasts and sub-chloroplast preparations oriented in polyacrylamid gel.

KAPLANOVÁ, M. and PARMA, L.: Study of fluorescence decay of clorophyll-a in model systems.

Extended abstracts of the lectures were published on pages 111—195 of the Conference Digest.

L. SZALAY

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